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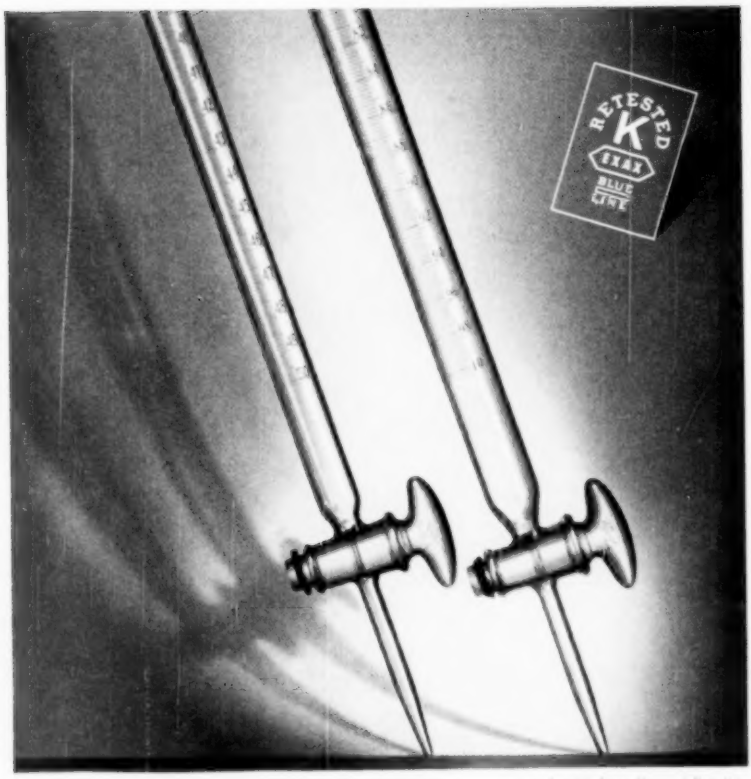

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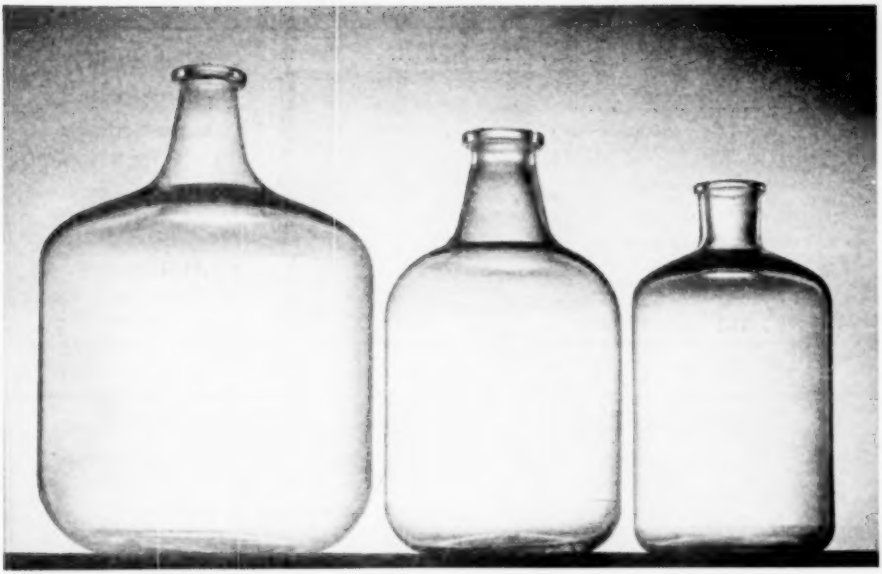

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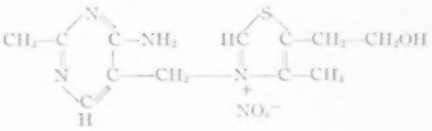
Pharmaceutical Studies with Thiamine Mononitrate*

By THOMAS J. MACEK, BEATE A. FELLER, and EDWARD J. HANUS†

Thiamine mononitrate, the salt formed from one mole of thiamine base and one mole of nitric acid, occurs as an anhydrous crystalline solid of low hygroscopicity, differing in this respect from thiamine hydrochloride. The stability of thiamine mononitrate was investigated in sterile solutions, compressed tablets, multivitamin capsules and dry-filled capsules. Assay data are given to show that thiamine mononitrate is more stable than thiamine hydrochloride in these preparations. The use of thiamine mononitrate in B complex capsules eliminated the need for an acidic stabilizer, the presence of which accelerated the decomposition of the pantothenic acid component.

THIAMINE HYDROCHLORIDE U. S. P is commercially synthesized as thiamine chloride hydrochloride and crystallizes from alcoholic-aqueous solutions as the hemihydrate (m. p. = 248–250° with decomposition) (1). The molar proportion of water of crystallization, however, is not constant during storage and may vary between one-half and approximately one, depending upon humidity conditions. A moisture content not exceeding 5 per cent of the solid is permitted by the U. S. P. (2). In spite of its variable moisture, crystalline thiamine hydrochloride as a solid is stable for extended periods at room temperature and at elevated temperatures even in contact with air. Aqueous solutions of thiamine hydrochloride in the *p*H range 2.5 to 4.5 likewise are stable for a considerable time even though precipitation sometimes occurs. In a number of food products and in certain pharmaceutical forms, particularly in mixtures with other vitamins, thiamine hydrochloride, however, has presented stability problems.

A compound which has been found to possess some interesting properties as regards stability, is the thiamine salt formed from one mole of thiamine base and one mole of nitric acid. This compound, called thiamine mononitrate, has the following structure:



Thiamine mononitrate is readily crystallized from water, leading to the production of a salt of

* Received January 12, 1950, from the Research Laboratories, Merck & Co., Inc., Rahway, N. J.

† We are indebted to the Microbiology Dept., Research and Development Division, Merck & Co., Inc., for the calcium pantothenate assays, and to the Gelatin Products Co., Inc., Detroit, for the preparation of the multivitamin capsules.

high purity which melts at 196–200° (with decomposition). The concentration of a saturated aqueous solution at 25° is 2.7 Gm. per 100 Gm. of water; the *p*H of such a solution is 6.8 to 7.1. Addition of a strong acid, e.g., hydrochloric or nitric, to solutions of thiamine mononitrate increases solubility by virtue of the ionic equilibria involved. For example, by the addition of sufficient hydrochloric acid to maintain a *p*H of 4, concentrations of thiamine mononitrate as high as 18.5 Gm. per 100 cc. can be attained at room temperature. The addition of a strong acid results in the neutralization of the free amino group, the (acidic) *p*K of which is known by direct titration to be 4.8.

Computed on the basis of molecular weights 1.00 mg. of thiamine mononitrate (mol. wt. 327.37) is equivalent to 1.03 mg. of thiamine hydrochloride. Thiamine mononitrate has a moisture content of about 0.1 per cent and a low hygroscopicity, differing in this respect from thiamine hydrochloride; it does not deliquesce even on exposure to 90 per cent relative humidity. These properties suggested the use of thiamine mononitrate as a more stable form of thiamine in pharmaceutical preparations.

EXPERIMENTAL

All assays for thiamine were done by the U. S. P. thiochrome method (3). The results are expressed in terms of the U. S. P. thiamine hydrochloride reference standard. During test periods the calcium pantothenate was determined microbiologically by the *L. arabinosus* method of Skeggs and Wright (4). The results are expressed as pantothenic acid. Samples were stored at room temperature protected from light and in an electric oven at 40° as indicated. The ampuls and vials used for sterile solutions conformed to the U. S. P. requirement for type I glass. Considering the reproducibility of the assay method and the uniformity of distribution of thiamine in the tablet and capsule preparations, variations in assay values within the limits of ±5% were regarded as insignificant.

Sterile Solutions.—The stability of thiamine mononitrate was studied in unadjusted solutions containing 10 mg. and 25 mg. thiamine mononitrate per cc., respectively, in distilled water. These solutions, filtered through Pyrex sintered glass Büchner-type filters, were subdivided into ampuls which were flame sealed. The ampuls were sterilized by autoclaving at 120° for twenty minutes. The solutions were assayed before and after autoclaving and again after various periods of storage at room temperature up to six months. The *p*H of the solutions was likewise determined at each assay interval. The *p*H of the solutions was in a range incompatible with the stability of thiamine from the start. As a result significant losses of thiamine occurred during storage as shown in Table I.

In view of this instability and also because higher concentrations of thiamine were more desirable, the study was repeated with aqueous solutions containing 100 mg. of thiamine mononitrate per cc., adjusted to *p*H 4.0 by the addition of hydrochloric acid. Table II shows the solutions which were prepared.

TABLE II.—COMPOSITION OF SOLUTIONS PREPARED

Per Cc.		Solution 1	Solution 2
100 mg.	Thiamine mononitrate	100 Gm.	100 Gm.
0.26 cc.	1 *N* Hydrochloric acid	260 cc.	260 cc.
5 mg.	Chlorobutanol U. S. P.	—	5 Gm.
	Distilled water, to make	1000 cc.	1000 cc.

The 1 *N* hydrochloric acid was added to approximately 600 cc. of distilled water (or distilled water containing the chlorobutanol) and the mixture was warmed to about 50°. The thiamine mononitrate was dissolved with stirring. The cooled solution was made to volume by the addition of distilled water.

Solution 1 was filtered and subdivided into 5 cc. ampuls and flame-sealed. The ampuls were sterilized by autoclaving at 120° for twenty minutes. Solution 2 was divided in half. The first portion (Part A) was filtered and was subdivided into 10 cc. ampul vials and stoppered with sleeve type rubber stoppers. The filled vials were sterilized by autoclaving at 120° for twenty minutes. The second portion (Part B) was sterilized by filtration through a micro-porous procelain candle and was aseptically subdivided into 10 cc. ampul vials and stoppered with sterilized sleeve-type rubber stoppers. All solutions were assayed initially for thiamine and again after various periods of storage at room temperature and at 40°. The stability data are summarized in Table III. At *p*H 4.0 thiamine mononitrate is stable in aqueous solution. The solutions were clear and free of precipitate, and had discolored to only a slight extent after storage at room temperature for one year. Samples of Solution 1 have also been stored at room temperature for as long as two years without precipitation.

TABLE I.—STABILITY OF THIAMINE MONONITRATE IN UNADJUSTED AQUEOUS SOLUTIONS

Theoretical Concn. per Cc.	Mg. per Cc. and % Loss			
	25 Mg.	*p*H	10 Mg.	*p*H
Before autoclaving	25.5	7.00	10.2	6.75
After autoclaving	23.6 (−6%)	6.15	9.77 (−2%)	6.20
After 1 mo. at RT	22.9 (−8%)	5.95	9.28 (−7%)	6.00
After 3 mo. at RT	22.2 (−11%)	5.75	8.75 (−13%)	5.65
After 6 mo. at RT	12.1 (−52%)	...	6.99 (−30%)	...

TABLE III.—STABILITY OF THIAMINE MONONITRATE IN AQUEOUS SOLUTIONS ADJUSTED TO *p*H 4.0

	Mg. per Cc. and % Loss					
	Solution 1		Solution 2			
			Part A		Part B	
Sterilization	Autoclaved	*p*H	Autoclaved	*p*H	Filtered	*p*H
Theoretical concentration	100.0	...	100.0	...	100.0	...
Initial assay	99.5	4.00	102.0	3.90	102.0	3.96
After 6 mo. at RT	96.8 (−3%)	4.00	94.5 (−7%)	3.84	102.0 (0)	3.90
After 12 mo. at RT	93.2 (−6%)	3.83	96.6 (−5%)	3.70	97.3 (−5%)	3.71
After 1 mo. at 40°	95.5 (−4%)	3.94	92.3 (−10%)	3.80	94.7 (−7%)	3.80
After 6 mo. at 40°	89.4 (−10%)	3.88	86.4 (−15%)	3.66	92.7 (−9%)	3.64

Compressed Tablets.—The stability of thiamine mononitrate was studied in B complex tablets. The preparations shown in Table IV, with and without ascorbic acid, respectively, illustrate the composition and method of manufacture, and are typical of several tablet formulations tested.

TABLE IV.—TYPICAL B COMPLEX TABLET FORMULAS

Per Tablet, Mg.		Tablet 1, Gm.	Tablet 2, Gm.
2.2	Thiamine mononitrate	2.2	2.2
	Sucrose		33.0
33.0	Ascorbic acid	33.0	
1.7	Calcium pantothenate	1.7	1.7
2.0	Riboflavin	2.0	2.0
0.5	Pyridoxine hydrochloride	0.5	0.5
10.0	Niacinamide	10.0	10.0
247.5	Sucrose	247.5	247.5
40.0	Cornstarch	40.0	40.0
13.1	Lubricants	13.1	13.1
350.0		350.0	350.0

A mixture of the calcium pantothenate, riboflavin, pyridoxine hydrochloride, niacinamide, sucrose, and cornstarch, all in fine powder, was granulated with mucilage of acacia. The granulation was dried at 45° and was screened 16 mesh. A portion of fines below 40 mesh was collected. The thiamine mononitrate and the ascorbic acid (or the sucrose added in place of ascorbic acid) were mixed with the fines, and then with the remainder of the granulation. The lubricant, which consisted of a mixture of talc, stearic acid, and calcium stearate, was sifted 80 mesh and was mixed with the granulation. The granulation mixture was compressed on a single-punch machine into standard cup tablets, 12/32 inch in diameter, each weighing 350 mg.

The tablets were stored in screw-capped amber bottles at room temperature and at 40° for stability testing. They were assayed for thiamine at the beginning of storage and were reassayed at various intervals. The data summarized in Table V indicate that thiamine mononitrate was stable in these compressed tablets containing other vitamins of the B complex.

Multivitamin Capsules.—A number of experiments performed to determine the stability of thiamine hydrochloride in commercially available multivitamin capsules indicated a general lack of stability of the thiamine component during extended periods of storage. Data illustrating this

TABLE V.—STABILITY OF THIAMINE MONONITRATE IN B COMPLEX TABLETS

	Mg. per Tablet and % Loss	
	Tablet 1	Tablet 2
Initial assay	2.14	2.12
After 6 mo. at RT	2.11 (−1%)	2.18 (+3%)
After 12 mo. at RT	2.13 (0)	2.15 (+1%)
After 1 mo. at 40°	2.04 (−5%)	2.08 (−2%)
After 3 mo. at 40°	2.04 (−5%)	2.11 (−1%)
After 6 mo. at 40°	2.17 (+1%)	2.13 (0)

are given in Table VI. Thiamine mononitrate was therefore tested for stability in multivitamin capsules. In order to directly compare the stability of thiamine mononitrate with thiamine hydrochloride, two experimental lots of capsules (capsules 1 and 2) were prepared for study. These differed only in the thiamine salt used. A third lot (capsule 3), prepared with thiamine mononitrate in a slightly different excipient, was also studied. The composition of these capsules is shown in Table VII.

The powdered vitamins were mixed with the melted excipient which consisted of hydrogenated vegetable oil, corn oil, and beeswax. The mixture was milled to a smooth paste and was filled into colored gelatin capsules on an automatic encapsulating machine. The filled capsules were hardened and dried.

The *p*H of the capsules was measured on an aqueous extract which was prepared by digesting 4 opened

TABLE VI.—STABILITY OF THIAMINE HYDROCHLORIDE IN MULTIVITAMIN CAPSULES

	Mg. per Capsule and % Loss			
	Sample A	Sample B	Sample C	Sample D
Label claim	1.50	1.50	1.50	1.32
Initial assay	1.41	1.41	1.48	...
After 2 mo. at RT	1.37 (−3%)	1.29 (−9%)	1.28 (−14%)	...
After 4 mo. at RT	1.15 (−19%)	1.23 (−13%)	1.34 (−10%)	1.15 (−13%)
After 6 mo. at RT	1.08 (−23%)	1.11 (−21%)	1.17 (−21%)	1.05 (−20%)
After 9 mo. at RT	1.01 (−28%)	1.00 (−29%)	1.18 (−21%)	1.00 (−24%)
After 12 mo. at RT	0.99 (−30%)	0.87 (−38%)	1.12 (−24%)	0.98 (−26%)

TABLE VII.—COMPOSITION OF MULTIVITAMIN CAPSULES

	Per Capsule		
	1	2	3
Thiamine hydrochloride	1.50 mg.	....	....
Thiamine mononitrate	....	1.50 mg.	1.50 mg.
Riboflavin	2.00 mg.	2.00 mg.	2.00 mg.
Niacinamide	20.00 mg.	20.00 mg.	20.00 mg.
Pyridoxine hydrochloride	0.10 mg.	0.10 mg.	0.10 mg.
Ascorbic acid	37.50 mg.	37.50 mg.	37.50 mg.
Calcium pantothenate	1.09 mg.	1.09 mg.	1.10 mg.
Vitamin A (from fish liver oil)	5000 U. S. P. units	5000 U. S. P. units	5000 U. S. P. units
Vitamin D (from fish liver oil)	500 U. S. P. units	500 U. S. P. units	....
Vitamin D (synthetic)	....	....	500 U. S. P. units
Excipients, to make	281 mg.	281 mg.	370 mg.
*p*H =	3.8	3.9	4.8

capsules with 10 cc. of distilled water at about 65°. The thiamine potency of each preparation was determined by assay at the beginning of the test period and again at regular intervals during storage at room temperature and at 40°. The capsules were stored in screw-capped amber bottles. In order to prevent sticking during storage at the elevated temperature, the capsules were dusted with talc before being placed in the oven. The stability of thiamine in these capsules is indicated by the assay data summarized in Table VIII.

Dry-Filled Capsules.—In order to evaluate the stability of thiamine mononitrate in dry-filled, hard gelatin capsules, two B complex mixtures were prepared. These contained thiamine hydrochloride and thiamine mononitrate, respectively, plus other B vitamins and lactose U. S. P. as a diluent, as shown in the formulas in Table IX.

TABLE VIII.—STABILITY OF THIAMINE HYDROCHLORIDE AND THIAMINE MONONITRATE IN MULTIVITAMIN CAPSULES

	Mg. per Capsule and % Loss		
Thiamine Salt =	Capsule 1 Hydrochloride	Capsule 2 Mononitrate	Capsule 3 Mononitrate
Initial assay	1.47	1.49	1.47
After 2 mo. at RT	1.45 (−2%)	1.44 (−3%)	1.46 (−1%)
After 4 mo. at RT	1.21 (−18%)	1.43 (−4%)	1.43 (−3%)
After 6 mo. at RT	1.10 (−25%)	1.34 (−10%)	1.47 (0)
After 9 mo. at RT	1.02 (−31%)	1.33 (−10%)	1.41 (−4%)
After 12 mo. at RT	0.99 (−33%)	1.32 (−10%)	1.46 (−1%)
After 6 wks. at 40°	1.01 (−31%)	1.37 (−8%)	1.48 (+1%)
After 3 mo. at 40°	0.72 (−51%)	1.15 (−23%)	1.48 (+1%)
After 6 mo. at 40°	0.43 (−71%)	1.01 (−32%)	1.45 (−2%)

TABLE IX.—DRY-FILLED CAPSULE FORMULATIONS

	Capsule 4, Mg.	Capsule 5, Mg.
Thiamine hydrochloride	1.000	....
Thiamine mononitrate	....	1.000
Riboflavin	0.500	0.500
Niacinamide	5.000	5.000
Pyridoxine hydrochloride	0.125	0.125
Calcium pantothenate	0.500	0.500
Lactose	316.650	316.650
Total	324.0	324.0

The powders were mixed with the lactose. Hard gelatin capsules were filled with each of the mixtures to contain 324 mg. per capsule.

For assay the contents of 20 capsules were emptied into a small mortar and the powder was thoroughly mixed by trituration. A weighed sample of the mixture was then taken for analysis. The capsules were assayed initially and at regular intervals during storage at room temperature and 40°. The data summarized in Table X indicate that thiamine mononitrate was more stable than thiamine hydrochloride in these capsules.

TABLE X.—STABILITY OF THIAMINE HYDROCHLORIDE AND THIAMINE MONONITRATE IN DRY-FILLED B COMPLEX CAPSULES

	Mg. per Capsule and % Loss	
	Capsule 4 (Cg. Thiamine HCl)	Capsule 5 (Cg. Thiamine Mononitrate)
Initial assay	0.95	0.99
After 2 mo. at RT	0.86 (−9%)	0.99 (0)
After 3 mo. at RT	0.78 (−18%)	0.98 (−1%)
After 6 mo. at RT	0.78 (−18%)	0.94 (−5%)
After 12 mo. at RT	0.73 (−23%)	0.97 (−2%)
After 1 mo. at 40°	0.45 (−53%)	0.96 (−3%)
After 2 mo. at 40°	0.28 (−70%)	0.98 (−1%)
After 3 mo. at 40°	0.26 (−73%)	0.98 (−1%)
After 6 mo. at 40°	....	0.90 (−9%)

TABLE XI.—OTHER DRY-FILLED B COMPLEX CAPSULE FORMULATIONS

	Capsule, Mg.				
	6	7	8	9	10
Thiamine hydrochloride	3.0	3.0	...	...	3.0
Thiamine mononitrate	...	...	3.0	3.0	...
Riboflavin	4.0	4.0	4.0	4.0	4.0
Pyridoxine hydrochloride	1.5	1.5	1.5	1.5	1.5
Calcium pantothenate	10.0	10.0	10.0	10.0	10.0
Niacinamide	30.0	...	30.0	...	30.0
Niacinamide hydrochloride[a]	...	40.0	...	40.0	...
Tartaric acid	...	...	...	...	0.65
Cornstarch, to make	324.0	324.0	324.0	324.0	324.0

[a] Forty milligrams of niacinamide HCl is equivalent to 30 mg. niacinamide.

TABLE XII.—STABILITY OF THIAMINE AND CALCIUM PANTOTHENATE IN DRY-FILLED B COMPLEX CAPSULES

	Mg. per Capsule and % Loss				
	6	7	8	9	10
Thiamine					
Initial assay	2.54	3.08	3.10	3.15	3.01
After 1 mo. at RT	1.58 (−38%)	3.04 (−1%)	3.11 (0)	3.08 (−2%)	2.84 (−6%)
After 3 mo. at RT	0.61 (−76%)	3.02 (−2%)	3.10 (0)	3.07 (−3%)	2.80 (−7%)
After 6 mo. at RT	0.44 (−83%)	3.02 (−2%)	3.10 (0)	3.00 (−5%)	2.80 (−7%)
After 1 mo. at 40°	0.82 (−68%)	3.05 (−1%)	2.95 (−5%)	3.15 (0)	2.91 (−3%)
After 3 mo. at 40°	0.53 (−79%)	....	2.95 (−5%)	3.02 (−4%)	2.86 (−5%)
After 6 mo. at 40°	0.44 (−83%)	3.00 (−3%)	3.00 (−3%)	3.00 (−5%)	2.85 (−5%)
Pantothenic Acid					
Initial assay	10.6	10.3	12.0	10.4	10.5
After 1 mo. at RT	10.6 (0)	10.0 (−3%)	12.0 (0)	9.1 (−13%)	9.7 (−8%)
After 3 mo. at RT	10.6 (0)	9.5 (−7%)	12.2 (0)	8.5 (−18%)	7.9 (−25%)
After 6 mo. at RT	10.6 (0)	7.3 (−29%)	12.3 (0)	6.8 (−35%)	6.9 (−34%)
After 1 mo. at 40°	10.7 (0)	6.9 (−33%)	11.9 (−1%)	6.0 (−42%)	6.5 (−38%)
After 3 mo. at 40°	....	2.4 (−77%)	11.5 (−3%)	2.2 (−79%)	1.7 (−84%)
After 6 mo. at 40°	10.6 (0)	....	11.0 (−9%)	1.3 (−88%)	0.3 (−97%)

In order to stabilize thiamine hydrochloride in B complex mixtures it has sometimes been the practice to add an acidic substance such as tartaric acid. Niacinamide hydrochloride has also been used in this way. Invariably the thiamine hydrochloride is stabilized but the rate of decomposition of other vitamin components of the mixture, notably calcium pantothenate, is increased. With thiamine mononitrate the addition of an acidic stabilizer becomes unnecessary. This was demonstrated by an experiment in which the thiamine and calcium pantothenate stability in a series of five dry filled B complex capsules was tested during storage at room temperature and at 40°. The capsule preparations were made containing variations of thiamine hydrochloride and thiamine mononitrate with niacinamide, niacinamide hydrochloride, and tartaric acid as shown in Table XI.

Hard gelatin capsules were filled with each mixture to contain 324 mg. per capsule. The contents of 20 capsules were mixed and sampled for each assay. The thiamine and pantothenic acid assay data for each of the capsules are summarized in Table XII.

SUMMARY

Pharmaceutical studies with thiamine mononitrate are reported. The addition of an acidic stabilizer in B complex capsules was found to be unnecessary when thiamine mononitrate was used. It was found to be more stable than thiamine hydrochloride in the multivitamin capsules and the dry-filled B complex capsules studied. Thiamine mononitrate was found to be stable in compressed tablets and in sterile aqueous solutions at *p*H 4.0. Some of the sterile solutions have been stored at room temperature for as long as two years without precipitation.

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The Action of Various Drugs on the Arterial Blood Flow of the Pregnant, Canine Uterus*,†,‡

By RAYMOND P. AHLQUIST

A method for studying the uterine blood flow and myometrial activity of intact experimental animals is described. It is shown, in the pregnant dog, that each uterine contraction, either spontaneous or induced by drug action, decreases the uterine arterial blood flow. The actions of fifteen pharmacological agents on uterine flow and myometrial activity are reported. The results indicate that drugs have an important effect on the uterine flow both by direct vascular and indirect myometrial action.

ALTHOUGH there is a voluminous literature on the actions of drugs on the myometrium of various species few studies on uterine blood flow have been reported. Reynolds (1, 2) has demonstrated the relationship between the duration of pregnancy and the uterine blood flow in rabbits and has described a method for measuring blood flow in the pregnant, simian uterus. A preliminary report from this laboratory (3) has described the blood flow in the postpartum, canine uterus.

In muscular structures such as the uterus the arterial blood flow is modified by the muscular contractions. Drugs, therefore, can influence this flow by two different peripheral mechanisms: by acting directly upon the blood vessels and indirectly by action on the myometrium. It is becoming more apparent that many drugs used in the treatment of uterine disfunction may not bring about their effects by direct myometrial action alone. Therefore, it is of importance to determine the actions of drugs on both the myometrium and the uterine blood flow. This present paper is concerned with a method for studying uterine blood flow in experimental animals and with a description of the actions of some of the common pharmacological agents on the arterial flow and myometrial activity of the pregnant, canine uterus.

METHODS

Pregnant dogs, judged near term, were anesthetized with intravenous sodium pentobarbital, 10–20 mg./Kg., following a subcutaneous dose of morphine sulfate, 10 mg./Kg. The uterus was exposed through a low midline incision and small water-filled balloons on woven silk catheters inserted into each horn through small stab wounds in the uterine wall and securely fastened with purse-string sutures. The balloon in the left horn was placed approximately in the mid-portion of the horn. The position of the right horn balloon was varied from animal to animal, being placed near the cranial end of the horn, in the mid portion, or very close to the fundus. After the administration of heparin, 10 mg./Kg., the left uterine artery was cannulated peripherally near the junction of the fundus and left horn. Blood from a centrally cannulated femoral artery was passed through a Shipley optically recording rotameter (4)[1] and into the cannulated uterine artery.

In several of the animals femoral arterial flow was also measured to serve as a control for some of the drug actions.

Recordings were made with an eight-channel optical recording instrument[2] constructed in this laboratory. The following Statham[3] transducers were employed: model P23-15G-255 for carotid pressure and arterial perfusion pressure from a side-arm in the rotameter system; model P6-5G-350, modified, or model P23-5G-300 for intra-uterine pressures; and in some of the animals the uterine wall tensions were recorded with one or more model G1-4-255 adapted to serve as isometric myographs. Fig. 1 illustrates one of the simpler records obtained.

Most of the experimental drugs were injected into the rotameter outflow in a volume of 0.1 cc. and in doses which in general had minimal effect on the arterial pressure. Supplementary heparin and pentobarbital were given as necessary during the course of the experiment.

RESULTS

The relationships between the spontaneous uterine activity and uterine arterial blood flow are presented in Table I. The maximum pressure developed in the intra-uterine balloons by spontaneous contractions was found to be about 25 mm. Hg. This is in contrast to the findings in humans (5) and postpartum dogs (3) in which much higher pressures are developed. Each contraction reduced the blood flow, in some cases as much as 50%. The degree of flow change was apparently related to the duration of pregnancy as shown in the table. This point, however, is still under investigation.

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‡ The following kindly furnished the drugs tested: Winthrop-Stearns (levo Arterenol, isopropyl Arterenol and Sympatol); Ciba Pharmaceutical Products (Priscoline and tripelennamine); Smith, Kline and French Labs. (Dibenamine).

[1] Obtainable from Mr. C. Wilson, 325 W. 42nd St., Indianapolis 8, Ind.

[2] The recording system was made up of two rotameter galvanometers and six type GA-1 galvanometers obtained from Heiland Research Corp., 130 E. 5th St., Denver, Colo.

[3] Statham Laboratories, Inc., 9528 Santa Monica Blvd., Beverly Hills, Calif.

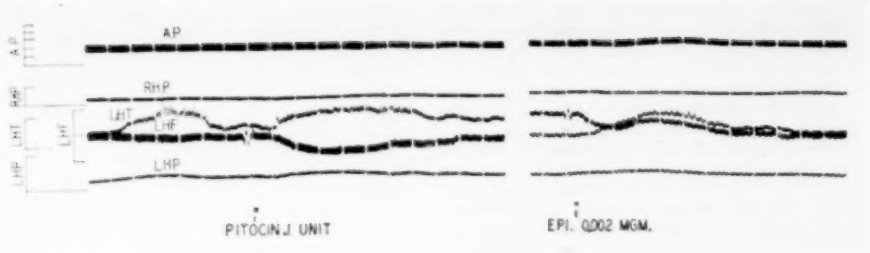

Fig. 1.—Relationship of uterine activity to uterine arterial blood flow.

Records from above downward: arterial pressure (AP) from carotid artery—calibration in units of 50 mm. Hg; right horn intra-uterine pressure (RHP)—calibration at 50 mm. Hg; tension in wall of left uterine horn (LHT)—calibration at 30 Gm.; arterial blood flow to left uterine horn (LHF)—calibration in units of 40 cc./min.; left uterine horn pressure (LHP)—calibration at 50 mm. Hg. Time breaks at thirty-second intervals.

The spontaneous contraction had little effect on the blood flow. The intra-arterial injection of Pitocin, 0.1 u./Kg., at the first signal produced a contraction and greatly diminished the blood flow. Epinephrine, 2 μg./Kg., at the second signal induced a contraction and diminished flow. Note that following the break in the record the flow and tension lines are inverted. The secondary increase in flow after the epinephrine is due to the rise in arterial pressure.

Each horn was found to contract at about the same rate but apparently independently of each other. Blood flow was modified only by the activity of the horn supplied by the artery in which flow was being measured; contractions of the contralateral horn had little effect on the flow. A more detailed analysis of the relationship between myometrial activity and uterine arterial flow will be published elsewhere.

Table II presents selected data which illustrate the effects of fifteen pharmacological agents on the blood flow and myometrial activity. To interpret the direct vascular action of those drugs which produce uterine contraction, reference to Table I must be made. It was found that the intra-uterine pressures developed by drug-induced contractions were no greater than those developed by maximal spontaneous contractions. Therefore, direct vasoconstriction was judged to be present if the decrease in flow produced by the drug was greater than the decrease in flow produced by a spontaneous contraction. Vasodilation was judged to be present if the flow increased or if the decrease in flow was less than that produced by a spontaneous contraction.

TABLE I.—RELATIONSHIP OF SPONTANEOUS UTERINE CONTRACTIONS AND BLOOD FLOW

Dog[a]	Weight, Kg.	Control Uterine Arterial Flow, Cc./Min.	Maximum Pressure During Contraction,[b] Mm. Hg	Decrease in Flow During Contraction, %
1	18	100	25	10
2	8	20	22	10
3	14	50	25	10
4	11	50	30	30
5	18	80	25	20
6	8	30	20	50
7	8	45	23	40

[a] Arranged according to apparent length of gestation as judged from the development of the fetus at autopsy. No. 1 longest, No. 7 shortest.

[b] In the horn corresponding to artery in which flow was measured.

The duration of action of several of the drugs on the myometrium and blood flow was found to be dependent upon the degree of blood flow change produced. Those which produced vasoconstriction had a prolonged effect due to the "trapping" of the drug by the constricted vascular bed. The vasodilators, on the other hand, had transient effects because of the rapid wash-through produced by the increased flow. This relationship was also demonstrated by the fact that the constrictors seldom produced general systemic effects after intra-arterial injection while the dilators usually produced a significant systemic effect.

Pitocin and Pitressin both produced vasoconstriction and myometrial stimulation. The former was more active as an oxytocic agent and less active as a constrictor. In some of the animals Pitocin produced vasodilation as indicated by a marked increase in blood flow. Most of the dogs were apparently hypersensitive to vasopressin since it has been shown (6) that the vasoconstriction produced by Pitocin is presumably due to its vasopressin content. Pitressin was more active as a constrictor and somewhat less active as an oxytocic. The oxytocic activity is not in accord with its comparative effectiveness on the isolated guinea-pig uterus but is in complete accord with its actions on the isolated rabbit uterus (7) and with its oxytocic activity in humans (8, 9).

Epinephrine produced marked vasoconstriction and a single uterine contraction followed by a period of uterine inhibition. The excitatory effects of epinephrine were diminished by the adrenergic blocking agents Dibenamine and Priscoline, but in no case were these effects completed, abolished or "reversed" by the doses tested. In contrast, the femoral vasoconstriction produced by epinephrine was usually changed to vasodilation by these blocking agents. These results indicate that the canine uterine vascular bed is only slightly affected by the sympathomimetic vasodilators. This was also demonstrated by the relatively slight vasodilation

produced by isopropyl-arterenol (10) in the uterine vascular bed as compared to its marked effect in the femoral vascular bed. Isopropyl-Arterenol was comparatively inactive on the myometrium, producing a questionable inhibition in most cases. This lack of effect can be explained in part by the rapid passage of the drug through the uterus.

Levo-Arterenol (11) produced about the same degree of vasoconstriction as epinephrine but was slightly less active as a uterine stimulant and much less active as a uterine inhibitor. Sympatol was less active than epinephrine as a constrictor and oxytocic and did not produce uterine inhibition in the animal tested. Ephedrine was quite inactive as compared to epinephrine, about 5,000 times more being required to produce a comparable effect.

Priscoline was found to be a very active vasodilator and myometrial stimulant. The dilation was more prolonged than the oxytocic effect. These results are in accord with its effects on other vascular beds and on other smooth muscle (12).

Dibenamine (13), administered intravenously, increased uterine flow somewhat but had no effect on the myometrial activity.

Acetylcholine and histamine were both active dilators and myometrial stimulants, the effects of both being very transient. Atropine prevented both effects of acetylcholine but had no effect on the actions of histamine. The myometrial action of histamine was blocked by tripelennamine but its dilator effect was practically unchanged. Tripelennamine itself was an active dilator and myometrial stimulant (14).

Heparin solution, atropine and pentobarbital sodium were active vasodilators when injected intra-arterially. Only the latter substance affected the myometrium, producing a transient inhibition. On intravenous injection these drugs had practically no effect on uterine flow or activity.

DISCUSSION

Because of species variation and anatomical dissimilarity these results cannot be applied directly to human pharmacology. However, it is clearly shown that drugs have an important effect on uterine blood flow through both direct vascular and indirect myometrial action. It is apparent, therefore, that the therapeutic value of drugs can be due to either or both effects. Further studies now in progress should yield useful information as to the precise mechanism of drug action on the uterus.

TABLE II.—EFFECTS OF VARIOUS DRUGS ON UTERINE ARTERIAL FLOW AND MYOMETRIAL ACTIVITY

Drug	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Dog 7
Pitocin	0.02 u.[a] −50%[b] C[c]	0.2 u. −80% S	0.1 u. −50% C	0.01 u. +50% C	0.02 u. −60% C	1.0 u. +30% C	0.5 u. −75% S
Pitressin	0.02 u. −70% C	0.2 u. −85% C	0.1 u. −50% C	0.01 u. −30% C	0.02 u. −60% C	1.0 u. −100% S	0.5 u. −90% C
Epinephrine HCl	2.0 μg. −92% CR	0.2 μg. −30% CR	2.0 μg. −85% CR	1.0 μg. −65% CR	5.0 μg. −100% CR	1.0 μg. −60% CR	1.0 μg. −100% CR
l-Arterenol HCl	2.0 μg. −90% Cr	...	2.0 μg. −90% Cr	...	...	1.0 μg. −50% Cr	...
Sympatol HCl	...	20 μg. −30% C	...	...	...	...	...
Ephedrine sulfate	...	0.1 mg. 0 C?	...	...	...	5.0 mg. −15% CR	...
Priscoline HCl	0.5 mg. +40% C	...	1.0 mg. +60% C	...	5.0 mg. +30%[d] C	...	...
Dibenamine HCl[e]	...	...	...	10.0 mg. +15%	...	...	20.0 mg. +10% O
Epinephrine after Priscoline or Diben.	2.0 μg. −90% CR	...	2.0 μg. −30% CR	1.0 μg. −40% CR	5.0 μg. −75% eR	...	1.0 μg. −60% eR
Isopropyl-Arterenol HCl	2.0 μg. ? O	20 μg. +10%[d] R?	10 μg. +10%[d] R?	100 μg. 0[d] R?	50 μg. +10%[d] R?	1.0 mg. +15%[d] C?	...
Acetylcholine chloride	10 μg. +30%[d] C	...	...	1.0 μg. −10% C	...	+	0.1 μg. +15% C?
Histamine phosphate	0.5 μg. +90% C	...	...	1.0 μg. +50%[d] C	...	...	...
Tripelennamine HCl	5.0 mg. +30% C	...	...	...	...	...	...
Histamine after tripelennamine	0.5 μg. +80% C?	...	...	...	...	...	...
Atropine sulfate	0.5 mg. +20% O	0.5 mg. +50% R?	0.5 mg. +10% O	0.5 mg. +50% O	0.5 mg. ? O	0.5 mg. +20% O	0.5 mg. +30% O
Heparin (1% soln.)	10 mg. +60% O	10 mg. +90% O	10 mg. +75% O	10 mg. +100% O	10 mg. +150% O	10 mg. +30% O	10 mg. +50% O
Pentobarbital sod.[f]	5.0 mg. +25% R	...	...	5.0 mg. +40% R	...	5.0 mg. +10% R	5.0 mg. +30% R

[a] Dose per Kg. injected intra-arterially.
[b] Maximum change in uterine blood flow as percentage of control flow.
[c] Myometrial response: C—normal type contraction; S—spasm or sustained contraction; R—relaxation or inhibition of normal activity; O—no measurable effect; ?—effect questionable; small letters indicate smaller effect.
[d] Following the primary increase in flow there was a secondary decrease due to a marked fall in arterial pressure.
[e] Ninety minutes after the intravenous administration of Dibenamine HCl.
[f] Injected for supplementary anesthesia.

SUMMARY

A method for the study of uterine blood flow and myometrial activity has been described. The relationship between uterine contractions and arterial flow in the pregnant, canine uterus has been demonstrated. The drugs tested by intra-arterial injection can be classified as:

Vasoconstrictor-oxytocic.—Pitocin, Pitressin, epinephrine, Arterenol, ephedrine and Sympatol.

Vasodilator-oxytocic.—Priscoline, acetylcholine, histamine and tripelennamine.

Vasodilator-inhibitor.—Isopropyl-Arterenol and pentobarbital sodium.

Vasodilator.—Dibenamine, heparin and atropine.

Epinephrine, Arterenol and ephedrine also produced myometrial inhibition following their oxytocic action.

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The Fallacy of the Square Root Sampling Rule*

By J. KEITH BORLAND†

It is demonstrated that the determination of sample size as the square root of the lot size may create a sense of false security. Lot size alone is shown to provide an incomplete basis for determining sample size, whether by "square root" sampling or percentage sampling. Sampling plans based on quantitative statements of the risks involved are recommended.

THERE is an increasing interest in the problem of properly sampling lots of pharmaceutical material for acceptance testing. The problem involves not only instruments and techniques but also sample size, which is the number of units taken from a lot for examination and testing. The literature of statistical methods is replete with detailed discussions of sampling theory (1–4). Noel (5) has given some pharmaceutical applications of statistical sampling plans, and Knudsen (6) has presented a valuable discussion on the special problem of sampling for sterility testing.

"How many samples should be taken from a lot for testing?" This question repeatedly arises where it is necessary to make decisions concerning the acceptability of lots from the evidence of small samples. In considering this question, one may define a lot as a group of units produced consecutively or concurrently under essentially similar conditions. One of the answers to the question has specified that the number of units sampled shall be the *square root of the total number of units in the lot*. The square root sampling rule has been used to a considerable extent by regulatory agencies of the government; it is the official A. O. A. C. method for sampling cereal foods, fruits and fruit products, butter, etc.; the U. S. P. XIII and N. F. VIII each set forth a schedule for sampling bulk drugs of animal or plant origin, which indicates that approximately the square root of the number of units in the lot should be sampled for acceptance testing. Many sampling plans in current use by industrial organizations are based on the number of units in the lot, thus

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samples are often taken by the square root rule or corresponding to an arbitrary percentage of the lot.[1]

Apparently the inference has been widely drawn that the square root sampling rule provides a fairly constant level of protection against failure to disclose defective material in a lot composed of numerous discrete units. Munch and Bidwell (7) stated that the "accuracy of sampling varies as the square root of the lot size." This statement was based on a formula for the probable error of the correlation coefficient, which contains an expression of the square root of a statistical universe. The statistical universe was considered to be equivalent to a lot of many discrete units. The quantities being correlated were not clearly identified, and the connection that a correlation study might have with sampling risks was not described. Nevertheless, the square root sampling scheme enjoys a wide area of acceptance because of its simplicity and perhaps to some extent because it is couched in mathematical terminology. However, mathematical considerations prove that sampling by the square root rule does not provide a constant level of protection. It is the purpose of this paper to indicate that a false sense of security may be engendered through its practice.

In the drug industry, specifications commonly require that all units in a sample must be found wholly acceptable prior to acceptance of the lot sampled. An example of this type of specification is found in testing for sterility of parenteral preparations. A container of parenteral solution may possess either of two alternative attributes: (*a*) it is sterile or (*b*) it is not sterile. Similarly, containers of basic chemicals or starting materials may be capable of two attributes: (*a*) acceptability or (*b*) nonacceptability. The method of determining the state of acceptability may involve, for example, compliance or lack of compliance with limits for purity. When nonacceptable containers are present in a lot of material, there is the problem of sampling and testing a sufficient number of the containers to detect the nonacceptable containers. It is often too expensive to examine all containers of the lot. In such cases, acceptability is based on the evidence available from samples of the lot.

For any given fraction defective in a lot, an operating characteristic curve shows the probability that such a lot will be accepted by the sampling plan.[2] A series of operating characteristic curves has been prepared to illustrate the various probabilities of accepting defective material when sampling by the square root rule. The symbols are defined as follows:

N = number of units in the lot.
p' = the fraction of the lot which is defective, also termed "lot fraction defective."
n = number of units taken from the lot at random for testing.
c = number of defective units present in the sample.
P_0 = the probability of finding no defective units in the sample (P_0 ranges from 0.0 to 1.0).

The operating characteristic curves may be used to estimate the probability (P_0) of accepting lots at p' lot fraction defective. The complement of P_0, $1 - P_0$, might be used as an expression of the probability of rejection. It should be noted that this applies only where the specification permits no defectives in the sample. $1 - P_0$ is the sum of the probabilities of finding c defectives in the sample where $c = 1, 2, 3 \ldots n$.

A hypothetical example is useful to illustrate the nature of operating characteristic curves. Consider a 10 per cent defective lot consisting of 100 units, from which a sample of 10 units is taken at random. Note that 10 is the square root of 100. What is the probability of finding no defective units in a sample of 10 units taken at random from this lot, which is actually 10 per cent defective? In other words, what is the probability of accepting a lot which is 10 per cent defective? The probabilities for several selected lot sizes may be read in Fig. 1 from the operating characteristic curves. In Fig. 1, the vertical axis at $p' = 0.10$ intersects the operating characteristic curve ($N = 100$, $n = 10$, $c = 0$) at $P_0 = 0.34$. Thus about one-third of the time, this sampling plan (10 samples taken from 100) would fail to disclose any defective units from a 10 per cent defective lot.

To illustrate the weakness of the square root sampling rule, operating characteristic curves have been plotted for several lots, the sizes of which are the squares of small whole numbers. Assume, for example, that the several lots ($N = 4$, 16, 25, 49, 100, 400, and 900) are all 10 per cent

[1] At a meeting of the Combined Contact Committee of the American Drug Manufacturers Association and the American Pharmaceutical Manufacturers Association held in April, 1949, the result of a questionnaire was reported which indicated that the square root system of sampling appears to be the one most generally used for testing the starting materials of drug manufacturing.

[2] Points on the operating characteristic curves are calculated from the expression:

$$P_0 = \frac{(N - C)!(N - n)!}{(N - C - n)!N!}$$

where N = number of units in the lot, C = the number of defective units in the lot, n = the number of units in the sample, P_0 = the probability of a sample containing no defective units, and ! = the factorial of the number preceding the sign.

defective. Here it should be noted that in several cases 10 per cent of the lot units involves the decimal fraction of a unit, i.e., where $N = 4$, 10 per cent of $N = 0.4$ of a unit. In spite of this mental hazard, it is very convenient to assume the same lot fraction defective for each lot because in this way the probabilities involved become directly comparable. The probabilities of accepting 10 per cent defective lots have been estimated from Fig. 1 and are given in Table I.

TABLE I.—PROBABILITIES (P_a) OF ACCEPTING 10 PER CENT DEFECTIVE LOTS OF VARIOUS SIZES (N), WHEN SAMPLING BY THE SQUARE ROOT RULE. LOTS ARE CONSIDERED ACCEPTABLE WHEN NO DEFECTIVE UNITS ($c = 0$) ARE DETECTED IN THE SAMPLE

N	n	c	p'	P_a
4	2	0	0.10	0.76
16	4	0	0.10	0.62
25	5	0	0.10	0.57
49	7	0	0.10	0.46
100	10	0	0.10	0.34
400	20	0	0.10	0.12
900	30	0	0.10	0.04

It can be observed in Table I that, for the lot sizes selected, the probabilities (P_a) of accepting 10 per cent defective lots vary from as high as 0.76 to as low as 0.04. In the case of small lot sizes, e.g., where $N = 4$ and $P_a = 0.76$, about three-quarters of the time a 10 per cent defective lot may be accepted. A lot of larger size, e.g., $N = 900$ and $P_a = 0.04$, will be accepted only about 4 per cent of the time. This wide range of probabilities demonstrates that the square root sampling rule definitely does not provide a constant level of protection.

In following the argument to its logical conclusion, it may be demonstrated further that the size of a lot has only a minor influence on the probability associated with the inference from sample to lot. Figure 2 gives operating characteristic curves for several of the same sample sizes (n) used in Fig. 1 but with the important difference that all lots are large enough to be considered infinite in size. As before, assume the lots are all 10 per cent defective. Probabilities (P_a) estimated from Fig. 2 for large lots are listed in Table II, together with the probabilities from lots of smaller size.

Table II demonstrates that, for a given sample size taken at random, a great increase in lot size, i.e., from a comparatively small number to practical infinity, makes a relatively small difference in the probability of finding no defectives in the sample. This is particularly striking in the case of sample size $n = 2$, when the lot size is increased from $4(n^2)$ to infinity (for practical purposes a lot of several thousand units is infinitely

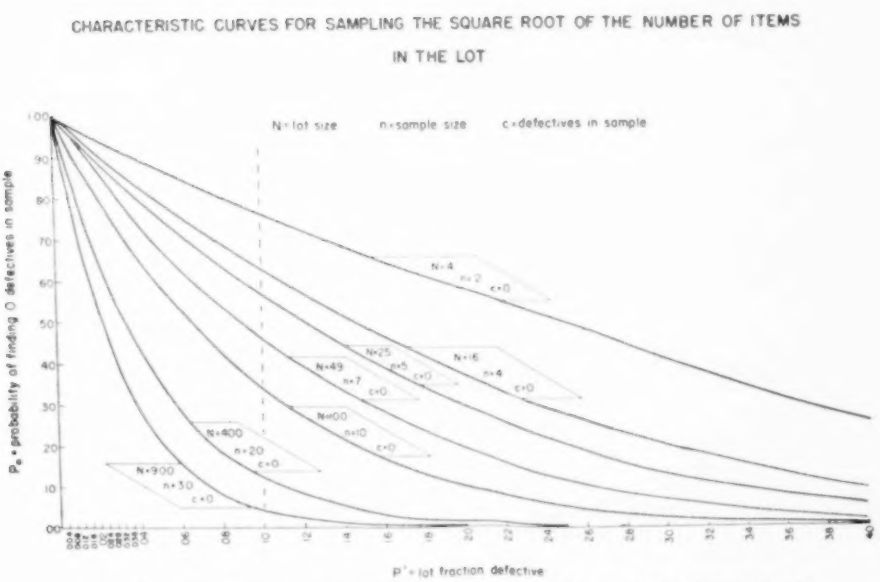
CHARACTERISTIC CURVES FOR SAMPLING THE SQUARE ROOT OF THE NUMBER OF ITEMS IN THE LOT
N= lot size n=sample size c=defectives in sample
N=4 n=2 c=0
N=25 n=5 c=0
N=16 n=4 c=0
N=49 n=7 c=0
N=100 n=10 c=0
N=400 n=20 c=0
N=900 n=30 c=0
Pa = probability of finding 0 defectives in sample
p' = lot fraction defective

Fig. 1.—Characteristic curves for sampling the square root of the number of items in the lot.
Operating characteristic curves for several sample sizes (n), which are numerically the square roots of the lot sizes (N), where P_a is the probability that there are no defective units in the sample.

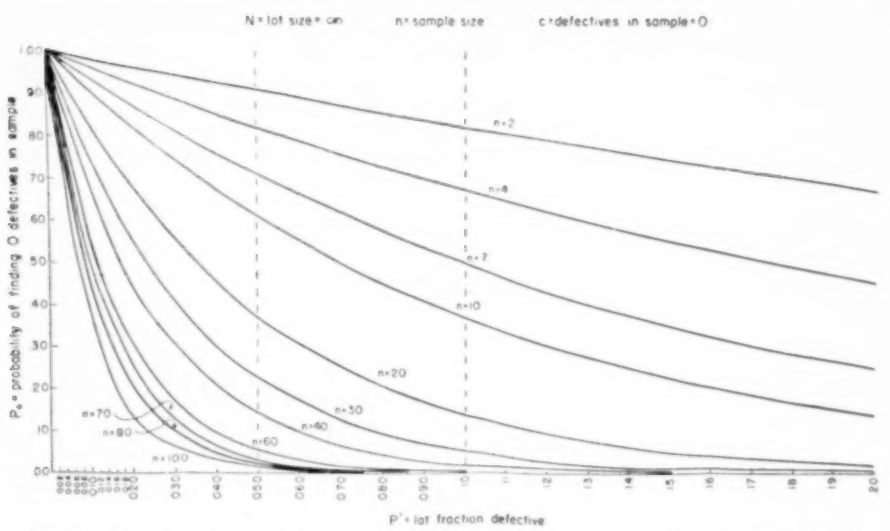

Fig. 2.—Operating characteristic curves for different sample sizes, assuming lot size is infinite. Operating characteristic curves for several sample sizes (n). The lot sizes (N) in each case are large enough to be considered infinite. P_0 is the probability that there are no defective units in the sample.

large) the probability of finding no defectives, in a 10 per cent defective lot, increases only from $P_0 = 0.76$ to $P_0 = 0.82$. This serves to emphasize the fact that the relation of sample size to lot size is comparatively unimportant. Both Tables I and II reveal that the probabilities are closely related to absolute sample size. Note that, as sample size increases from $n = 2$ to $n = 30$, the probability of finding no defectives decreases and lot size has little influence.

TABLE II.—PROBABILITIES OF ACCEPTANCE FOR SELECTED SAMPLE SIZES (n) FROM LOTS OF FINITE SIZE (n^2), AND FROM LOTS OF MUCH LARGER SIZE

Sample Size, n	P_0 for Lot Size = n^2	P_0 for Lots of Very Large Size
2	0.76	0.82
4	0.62	0.68
7	0.46	0.50
10	0.34	0.37
20	0.12	0.13
30	0.04	0.05

Having indicated the weakness of basing sample size on lot size, it should be noted that satisfactory sampling plans are readily available, but on a different basis. Well-designed sampling plans require quantitative expressions of acceptable and nonacceptable lot quality and of the risks involved of making wrong decisions regarding lot quality. These risks cannot be reduced to zero but they can be made reasonably low. Sampling plans may be "custom built" to provide the most efficient protection at selected levels of risk.

Figure 2 may be used in a limited way to determine sample size for attribute testing, i.e., in making the choice between two distinct alternatives which may be designated specifically as acceptable or nonacceptable, sterile or contaminated, black or white, hot or cold, etc. It will be recognized, of course, that the difference between the two categories needs only to be great enough to be distinguished infallibly. That is, if temperature is the attribute under test, the material must be definitely hotter or unquestionably colder than some standard or reference temperature. The case of intermediate samples which are confusingly close to the borderline presents other problems requiring special attention. With these qualifications Fig. 2 indicates the sample size to be taken from comparatively large lots on the basis of the risk one is willing to take. For example, assume that a manufacturer must determine the acceptability of a lot consisting of thousands of units. If one grants that 100 per cent perfection is an unattainable ideal, a small proportion of defective units must be tolerated in

some cases.[3] For economic reasons one may assume, for example, that not more than 5 per cent of defective units may be tolerated in the lot. In Fig. 2, this is indicated by the vertical dotted line at $p' = 0.05$. The question now arises as to the level of risk to be taken that the sample from a 5 per cent defective lot will fail to reveal defective units actually present in the lot. The selection of risks, will depend on costs: the cost of testing the samples, that of obtaining the samples, or cost of the samples themselves may all be substantial. There are also the costs of customer dissatisfaction with a defective product. The cost usually increases as the risk decreases. In order to select intelligently the most economic risk level it is necessary to know the risks related to different sample sizes. An example of such information is given in Table III where $p' = 0.05$ lot fraction defective and P_0 = the probability of disclosing no defectives in the sample.

TABLE III.—PROBABILITIES (P_0 ESTIMATED FROM FIG. 2) FOR SEVERAL SAMPLE SIZES (n) FROM A LOT OF INFINITE SIZE WHICH IS 5 PER CENT DEFECTIVE

n:	2	4	7	10	20	30	40	60	80	100
P_0	0.91	0.81	0.71	0.61	0.37	0.22	0.14	0.06	0.02	0.01

Referring to Table III, if only two samples were taken from a large lot which contains 5 per cent of defective units, 91 per cent of the time no defective units would be disclosed by the sample. Such odds may be intolerable, but to reduce them, the sample size must be increased. If one is willing to risk failure to disclose defective units only 1 per cent of the time on the average, a sample size of 100 units must be examined. The cost of examining a large sample is the cost of reducing the risks to a low level.

The extreme cases have been presented to give emphasis. In some cases, the mathematical risks may be diminished subjectively by collateral information. For example, in considering the sterility of a lot of parenteral solutions, the testing of a small sample only gives information concerning a possible gross contamination, however, knowledge of the efficacy of the steam sterilizer gives much added assurance of sterility and serves to reduce the risks somewhat.

[3] For the peace of mind of the manufacturer of sterile products it is fortunate that much ancillary information may be drawn upon to bolster the result of a sterility test since it is scarcely conceivable that one would willingly take any risk of distributing a product for which there was any measurable probability that it was contaminated with viable organisms.

SUMMARY

The square root sampling rule, which has attained semiofficial status and has been a basis for sampling in both industrial and regulatory control, does not provide a constant level of protection against accepting defective material.

Operating characteristic curves demonstrate that the square root sampling rule may create a sense of false security in attribute sampling.

Lot size is shown to be much less important than absolute sample size in controlling risks of error.

Probabilities of acceptance are given for lots varying in size from 4 (2^2) to 900 (30^2).

Families of operating characteristic curves are shown to be useful in designing attribute sampling plans.

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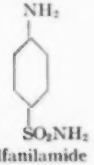
Substituted Ammonium Saccharins for Nasal Medication*

By WILLIAM F. HAMILTON and FREDERICK M. TURNBULL†

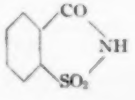
Saccharin reacts with amines to form salts which are substituted ammonium saccharins. Fourteen of these salts have been prepared, and forty-one of them were formed in aqueous solution and tested for bacterial growth antagonism against *S. aureus*. Nasal medication was formulated using 0.5 per cent of the desoxyephedrine salt and 0.02 per cent of the laurylamine salt as the active ingredients. This preparation was tested pharmacologically and clinically and was found to be safe and effective.

SACCHARIN (*o*-sulfobenzimide) has been used as a sweetening agent since its discovery in 1879 by Fahlberg and Remsen (1). It has been the subject of many investigations from chemical, bacteriological, and clinical aspects. The wealth of information which has been published on it is reviewed by Suter (2) who concludes in his discussion of the physical and physiological properties of the compund that "there is apparently no case on record where a human illness has been ascribed to the use of saccharin in normal amounts." He also says that saccharin has been reported to have a marked bactericidal action, greater than that of phenol, but that this seems to have been somewhat exaggerated.

Inspection of the chemical structure of saccharin reveals that it is somewhat similar to sulfanilamide:



Sulfanilamide



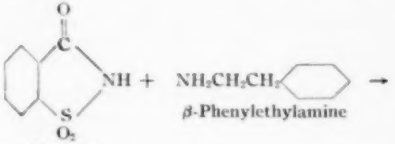
Saccharin

and both compounds will form salts with amines. The remarkable physiological properties of the N^1 amine salts of the sulfonamides have been carefully investigated (3, 4) and widespread clinical use of these compounds has resulted from their beneficial effects when applied topically for relief and control of secondary invaders in upper respiratory infections. By analogy, the salts of saccharin might be expected to have pronounced physiological activity and possibly some bacterial growth antagonistic properties provided that physiologically active amines were used for their preparation.

A few amine saccharinates are known, Givaudan (5) having prepared the methyl and ethyl ammonium saccharins about fifty years ago. Some thirty years later Mannessier-Mameli (6) prepared several arylammonium saccharins for the first time, notably the aniline, toluidine, and several related compounds. No pressor amine saccharinates have been prepared or investigated, nor had these salts been studied for bacterial growth antagonism.

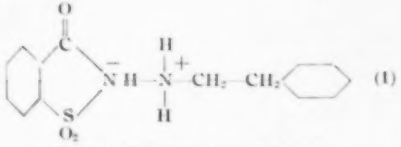
EXPERIMENTAL

The fundamental chemistry involved in the preparation of the amine saccharinates is clear. It is the addition of one molecule of the alkaline amine to one molecule of the acidic saccharin. It is a straightforward neutralization reaction wherein the two N atoms may be regarded as becoming ionically bonded, one being trivalent and the other (the amino N) being pentavalent. For an example, see Eq. 1.



Saccharin

β-Phenylethylamine



(1)

β-Phenylethylammonium saccharin

Salt formation occurs in the same manner by double decomposition between a saccharin salt and an amine salt to form the amine saccharinate. For example, sodium saccharin reacts with desoxyephedrine hydrochloride to form desoxyephedrine saccharinate and sodium chloride. If the reaction is carried out by dissolving the materials in absolute methanol, addition of diethyl ether will precipitate the sodium chloride, which may then be separated

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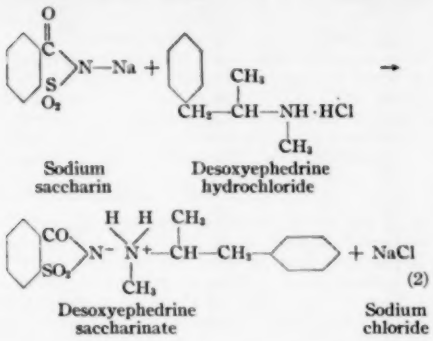
† The authors are indebted to Dr. A. G. R. Strickland for carrying out the bacteriological and ciliary motility experiments which are reported.

from the solution by filtration. The reaction which occurs is shown in Eq. 2.



Sodium saccharin — Desoxyephedrine hydrochloride

Desoxyephedrine saccharinate — Sodium chloride (2)

Salt formation occurs equally well with primary, secondary or tertiary amines. In case the acid and base are reacted alone, the amine saccharinate may be formed by simply melting the two together or reacting them in aqueous solution or suitable organic solvent.

In general the new salts are very difficult to crystallize although theoretically they might be expected to be white, crystalline solids. Usually they remain amorphous, waxy, or resinous and even after standing as long as a year do not crystallize. They are usually very soluble in water forming neutral or slightly acidic solutions, and in general are relatively stable materials. Some of the salts are very hygroscopic, particularly the Neo-Synephrine and *n*-hexylamine compounds. The characteristics of saccharinates which have been made are indicated in Table I.

BACTERIOLOGICAL

To evaluate the growth antagonism of various amine saccharinates it was not necessary to isolate the pure salts, as they could be prepared in aqueous solution by neutralizing the calculated amount of saccharin to make any given concentration of the amine saccharinate. The potency of the salt solution was tested against *Staphylococcus aureus* on agar plates.

A filter disk 12.5 mm. in diameter was saturated with a 10% solution of the salt to be tested, or a less concentrated solution in case the solubility was lower than 10%. Growth inhibition was noted by measuring the diameter of the clear or hazy circle which formed around the disk in case growth was impeded. The test results are tabulated in Table II.

Forty-one different amine saccharinates were tested in this manner and the following observations seem applicable to the experimental results:

1. Saccharin salts of alkyl amines having three or more carbon atoms in the hydrocarbon chain are potent growth inhibitors for *S. aureus*.
2. The presence of hydroxyl groups in the amine radicles of the saccharinates decreases growth antagonism.

These observations are limited to this series of tests because it is demonstrated in Table III that the size of the ring of growth inhibition is not directly related to the threshold concentration of growth inhibition as determined in tube culture tests against the same organism.

Tube culture tests proved to be a much more satisfactory method for evaluation of growth inhibition when quantitative information was desired. *S. aureus* was used in broth tubes which were always kept constant in concentration. Only the proportion of the salts to be tested was varied. Determinations were usually run in duplicate and the results were not considered final until they had been checked at least three or four times and on different days. As controls, unmedicated samples of the broth innoculated with the bacteria accompanied each series of tests. Growth inhibition was judged by the clarity of the test solution in comparison with the controls, and where a more exact comparison was desired, the unknown was compared to the control which was diluted to the same apparent turbidity.

When it was desired to know whether the medication was merely inhibiting growth and whether bacteria in the solution were still alive, samples from the clear culture tubes were transferred to sterilized unmedicated broth. If growth did not occur on repeated testing the salt was considered bactericidal at the concentration tested.

Some culture tubes were used to determine the effectiveness of laurylammonium saccharin against the growth of *Streptococcus hemolyticus* and *S. viri-*

TABLE I.—CHARACTERISTICS OF VARIOUS SACCHARINATES

N-Saccharin Salt of	Description	Remarks
n-Octadecylamine	Soft white waxy powder	M. p. ca. 82°
n-Dodecylamine (Laurylamine)	White wax-like solid	M. p. ca. 60°
Di-*n*-decylamine	Long yellowish needles	M. p. ca. 43°
n-Decylamine	White wax-like solid	.....
n-Heptylamine	Clear yellowish resin	.....
Di-*n*-heptylamine	Viscous gum	.....
n-Hexylamine	White, waxy crystals	Hygroscopic m. p. ca. 37°
n-Butylamine	White wax	.....
Di-*n*-butylamine	Wax-like crystals	.....
Tri-*n*-butylamine	Yellowish viscous liquid	.....
l-Ephedrine	Clear yellow resin	Conchoidal fracture
dl-Desoxyephedrine	Light yellow resin	Conchoidal fracture
dl-Amphetamine	Brittle light yellow resin	Conchoidal fracture
l-Neo-Synephrine	White crystals	M. p. ca. 63° Hygroscopic

TABLE II.—TABULATION OF TEST RESULTS

N-Salt of Saccharin with	Concentration, %, w/v	Diameter of Area, Mm. Clear	Hazy
Phenylamine (Aniline)	10	Nil	Nil
Phenylmethylamine	10	17.5	...
β-Phenylethylamine	10	17	22
Phenylisopropylamine	10	16.5	...
Phenylisopropylmethylamine	10	16.5	...
4-OH Phenylethylamine	10	Faint	...
Phenylisopropanolamine	10	14	...
Phenylisopropanolmethylamine	10	14	...
3-OH Phenylisopropanolamine	10	14–	...
3-OH Phenylethanolmethylamine	10	Trace	...
Pyridine	10	15	...
Cyclohexylamine	10	15	26
n-Hexylamine	10	19	...
Di-n-hexylamine	10	17	...
Monoethylamine	10	Nil	Nil
Diethylamine	10	Nil	Nil
Triethylamine	10	Nil	Nil
Monoisopropylamine	10	...	16
Mono-n-butylamine	10	...	17
Di-n-butylamine	10	...	16.5
Tri-n-butylamine	10	...	18
Mono-n-amylamine	10	...	16.5
Mono-n-heptylamine	2.5	17	...
Di-n-heptylamine	0.5	18	...
2-Aminoheptane	10	20	28
Mono-n-octylamine	1.5	16.5	...
Di-n-octylamine	0.1	15	...
Mono-n-decylamine	0.4	18.5	...
Di-n-decylamine	0.01	Nil	Nil
Mono-n-dodecylamine	0.1	14	...
Mono-n-octadecylamine	Emulsified	...	...
9-Aminoacridine	Satd.	Nil	Nil
Triethanolamine	10	Nil	Nil
4-Amino-2-butanol	10	Nil	Nil
2-Amino-2-methyl-1,3-propanediol	10	Nil	Nil
β-Diethylaminoethanol	10	Nil	Nil
α-Naphthylamine	Solidified	...	...
Methoxyethylamine	10	Nil	Nil
2-Aminothiazole	Decomposed	...	...

TABLE III.—TUBE CULTURE TESTS, *S. aureus*

Saccharin Salt of	Concentration, %, w/v	Growth Inhibition	Remarks
Desoxyephedrine	1.0	Excellent	.....
Desoxyephedrine	0.5	Poor	.....
Desoxyephedrine	0.5	Excellent	The 0.05% solution
with 1:25	0.25	Bactericidal	was bactericidal
Laurylamine	0.1	Bactericidal	to *S. viridans* and
	0.05	Good 80%	*S. hemolyticus*
Neo-Synephrine	1.0	Excellent	.....
Neo-Synephrine	0.5	Excellent	.....
Neo-Synephrine	0.1	Some	.....
n-Decylamine	0.05	Bactericidal	.....
n-Decylamine	0.01	Partial	.....
Laurylamine	0.005	Bactericidal	This salt was the
Laurylamine	0.002	Bacteriostatic	best growth an-
Laurylamine	0.001	Partial	tagonist tested
Octadecylamine	0.005	Threshold	.....
Cyclohexylamine	1.0	Excellent	.....
Cyclohexylamine	0.5	Good	.....
Cyclohexylamine	0.1	Some	.....
2-Aminoheptane	1.0	Excellent	.....
2-Aminoheptane	0.5	Good	.....
2-Aminoheptane	0.1	Some	.....

dans.[1] Strains of these fastidious organisms were cultured in the usual beef heart and brain infusion, whereas *S. aureus* was cultured in the usual peptone-yeast-dextrose nutrient.

At a concentration of 0.001% laurylammonium saccharin is bactericidal to *S. viridans* and *S. hemolyticus*. Independent studies[2] confirmed the fact that desoxyephedrine saccharinate with 1:25 laurylammonium saccharin is also bactericidal to *Pyocyaneus* strains, *B. coli*, and Friedlander's bacillus—pathogens commonly found in nasal secretions.

Nasal Medication

Using the two most promising of the new compounds, and after preliminary trials of thirty-two different mixtures, the following final formula was adopted[3] with active ingredients: Desoxyephedrine saccharinate, 0.50%, *w/v;* and laurylammonium saccharin, 0.02%, *w/v*, in an 0.9% sodium chloride solution, buffered to *p*H 6.5 by the addition of mixed sodium biphosphate and disodium phosphate, 0.1%, and flavored with celery extract.

The desoxyephedrine compound is the vasoconstrictive agent, while the laurylammonium saccharin is the bactericide in the preparation.

The finished solution after filtration through a fine asbestos plate is practically clear and water white. It is stable to light and heat and can be autoclaved without apparent loss in potency. Samples set on the roof in the summer, exposed to air and sunlight, remained for over three months with no apparent change in *p*H or color.

The preparation appears self sterilizing. Five streaks of each of five different batches were made on five nutrient agar plates with 1% added glucose, and were held for twenty-four hours at 24–25°. No growth of any kind occurred. After forty-eight hours of incubation at 38° there was still no growth. Confirming tests made in triplicate on ten different bottles,[4] subculturing to thioglycollate medium and Sabauroud maltose agar, using controls and *S. aureus, C. Novyi*, and *C. Albicans* as test organisms showed each bottle was sterile.

The ciliary motility approximated fifty minutes, as shown by studies on excised rabbit tracheal rings at 24–26°. Thus, the medicament had little effect upon the cilia and so there would be no derogatory effect anticipated upon nasal mucosa.

The toxicology of the medicament was studied[4] and the findings may be summarized as follows:

Acute Toxicity.—(*a*) The LD_{50} for mice was found to be:

Route	Dilution of Medication	Active Ingredients Contained per Dose: Desoxyephedrine Saccharinate, Mg./Kg.	Active Ingredients Contained per Dose: Laurylammonium Saccharin, Mg./Kg.
Subcutaneous	1:3	12.5	0.5
Oral	1:1	25	1.0
Intraperitoneal	Undiluted	60–70	24–28

(*b*) The subcutaneous LD_{50} for mice of a solution of desoxyephedrine saccharinate alone was 12.5 mg./Kg.; equivalent to the amount contained in the LD_{50} dose of the medication. A solution of 2 mg./Kg. of laurylammonium saccharin did not produce any symptoms when injected subcutaneously into mice.

(*c*) The intraperitoneal LD_{50} of the medicament in mice was found to be of the same order of magnitude as for *d*-desoxyephedrine as reported in the literature (7).

(*d*) Laurylammonium saccharin, 2 mg./Kg. subcutaneously in mice was found to be nontoxic. This dose is of the order of magnitude of 500 times greater than the recommended intranasal human dose, i.e., 1 ml. of medication containing 0.2 mg. of laurylammonium saccharin per 50 Kg. average human.

(*e*) Twenty hourly doses during two days, fed to mice, was found to be nontoxic. Each dose administered was equal to the recommended dose for an average 50 Kg. human.

Chronic Toxicity.—Daily single doses of medication 150–200 times greater than the recommended single human dose for an average 50 Kg. human did not reveal any gross or microscopic toxic effects when administered intranasally to guinea pigs, and subcutaneously and orally to rats.

The pressor effect of the medicament was studied in cats by the same laboratory.[4] Intranasally, the instillation of 1 ml. of the medicament did not alter the blood pressure or respiratory rate. In contrast, intranasal instillation of 0.5 and 1.0 ml. of 0.5% Neo-Synephrine hydrochloride resulted in a blood pressure rise of 60% with near cessation of respiration in one instance; a 23% blood pressure rise and a 42% rise in respiratory rate in another instance. Intravenously in cats, the medicament was diluted to contain 0.1 mg. desoxyephedrine saccharinate per injection, and caused an average blood pressure rise of 6% in seven trials, whereas Neo-Synephrine hydrochloride at the same dilution (0.1 mg. per injection) caused a blood pressure rise of 31% in three instances, 47% in one instance and 51% in one instance.

CLINICAL OBSERVATIONS

Clinical observations were made on over thirteen hundred cases. The preparation was used in office treatments by packs and tampons (8) as well as for home treatments by the patients themselves (9). The findings may be summarized as follows:

1. The degree of shrinking appeared to be consistently in the range of 3+ to 4+ (satisfactory) without the appearance of paleness found in overshrinking.

2. The duration of decongestion—the nose remained open for a period of a few hours on the first day of an acute head cold, and for eight to thirteen hours in cases of chronic congestion as in chronic sinusitis.

3. Reactions: (*a*) Local. There was noticeable absence of stinging, irritation, and returgescence; (*b*) general, central nervous system. Nervousness, excitability, restlessness, and sleeplessness were noticeably absent. The preparation was used in those susceptible to ephedrine reactions, in pregnancy,

[1] Strains of *S. viridans* and *S. hemolyticus* were obtained through the courtesy of the Los Angeles Milk Commission Laboratory at the University of Southern California. The *S. viridans* was penicillin-resistant and had been cultured from a serious case of recurrent bacterial endocarditis at the Los Angeles County General Hospital.
[2] Bensinger & Schlessinger, New York.
[3] "Rhinalgan," Doho Chemical Corporation, New York.
[4] Biological Laboratories, Inc., Brooklyn, N. Y.

in children, and in infants without unfavorable results; (*c*) Cardiovascular. No rise in blood pressure was noted in normal or hypertensive cases in 47 patients.

The studies indicate the medication is a conservative and efficacious nasal decongestant.

SUMMARY

1. Fourteen amine-saccharin salts have been prepared.

2. Forty-one amine-saccharin salts have been tested in aqueous solution for antibacterial activity.

3. Solutions of the saccharin salts of desoxyephedrine and laurylamine were particularly promising for nasal medication.

4. These salts were formulated into nasal medicament and were tested bacteriologically, pharmacologically, and clinically.

5. The preparation appears safe and effective.

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The Sympathomimetic Depressor Agents: A Review*

By RAYMOND P. AHLQUIST and JAMES P. HENSEN

IT HAS long been known that epinephrine can produce a depressor effect in experimental animals under certain conditions. A transient fall in arterial pressure often results from very small intravenous doses in cats and dogs; a secondary fall in pressure commonly occurs following the pressor response to larger intravenous doses; and following the administration of an adrenergic blocking agent epinephrine will produce a marked fall in pressure in most species. Just as the pressor actions of the sympathomimetic agents can be modified by changes in chemical structure so also can the depressor effects be enhanced or diminished by structural changes.

The recent clinical employment of some of the depressor sympathomimetic agents in the treatment of bronchial asthma and peripheral vascular disease has stimulated interest in this class of agents. This review has been prepared because other reviews on the sympathomimetic agents have been chiefly concerned with their pressor effects (15, 29, 30), with a particular group of amines (33), or with other pharmacological or biochemical aspects (10).

* Received January 12, 1950, from the Department of Pharmacology, Medical College of Georgia, Augusta, Ga.

For the purpose of this review a true sympathomimetic depressor agent should have the following qualifications: (*a*) proper chemical structure; (*b*) a depressor effect produced by peripheral vasodilation rather than cardiac depression; and (*c*) this depressor effect should be enhanced or unchanged by the typical adrenergic blocking agents and not prevented by atropine or the antihistaminic agents. The majority of the compounds listed below qualify only on the first point for several reasons. Most of the reported experimental studies on the sympathomimetic agents have been directed toward finding new pressor agents. Therefore, those which have shown depressor effects have not been studied or reported in detail. Also, most published results have been based on arterial pressure changes alone, and the peripheral vascular effects have not been directly determined. The amines which have been demonstrated by direct blood flow determinations to be peripheral vasodilators are indicated by an asterisk in the tables.

This review will present in tabular form the depressor activity and the acute toxicity of the sympathomimetic depressor amines. Other pharmacological effects are not presented since not enough comparative studies have appeared in the

literature. A recent review by Lands (33) has presented the comparative effects of a number of these agents on the bronchi, intestine, and uterus in addition to their effects on the cardiovascular system.

In this presentation preference has been given to those references which include results obtained on series of compounds rather than studies of individual compounds. The following references are included because the compounds considered therein are presumably depressor but not enough information is given to include in the tables, or because they deal with other aspects of the depressor amines: (11, 18, 20, 24, 39, 42, 46, 47, 50–53, 56).

Four types of depressor action can be recognized; (*a*) that which occurs occasionally depending in part on the dose tested; (*b*) that which occurs as the usual primary effect of the amine; (*c*) that which appears secondary to a primary pressor effect; and (*d*) that which appears following the administration of an adrenergic blocking agent. Since the species tested and anesthesia employed are important factors with any cardiovascular agent, these are also given when possible.

Table I lists the aliphatic amines reported to have depressor activity. The amines of lower molecular weight are very questionable sympathomimetic agents, and although several have been shown to have depressor action (54) these are not listed. A large series of aliphatic amines have been described by Swanson and Chen (48, 49) but none were found to have depressor action.

Table II lists the phenylethylamine derivatives in which substitutions have been made on the *alpha* and *beta* C atoms and on the N. In Table III are listed the phenylethanolamine derivatives with substitutions being made on the *alpha* C atom or N. Table IV gives the hydroxyphenylethanolamines and Table V presents the hydroxyphenyl-, methoxyphenyl-, cyclohexyl-, and cyclopentyl-ethylamine derivatives.

Another series of depressor agents which are partly sympathomimetic in action are the imidazolines (28, 44). The 2-alkyl imidazolines (methyl to heptyl) are all depressor agents with a minimal effective dose of about 10 mg./Kg. in dogs. The 2-cyclohexyl derivative has the same order of activity as the alkyl derivatives, the 2-cyclohexenyl compound is more active, and 2-benzyl imidazoline (Priscoline) is about 100 times more active. Substitutions on 2-benzyl imidazoline, for example, N-methylbenzyl imidazoline or 3,4,5-trimethoxybenzyl imidazoline, produce pressor agents, while substitutions on the methylene bridge only diminish the depressor activity.

TABLE I.—ALIPHATIC AMINES

	Amine	Type[a]	Animal[b]	Dose,[c] Mg./Kg.	Response,[d] Mm. Hg	Ref.	Acute Toxicity[e] Mg./Kg.	Ref.
1	*n*-Butyl amine	Prim.	C-2-Ure.	7.5 (5–10)	−20% (−30 − 11)	(54)	...	...
2	*sec.*-Butyl amine	Occas.	C-4-Ure.	10 (5–15)	− 0% (−15 + 18)	(54)	...	...
3	Isobutyl amine	Prim.	C-3-Ure.	10 (2–20)	− 4% (−30 − 0)	(54)	...	...
4[f]	2-Amino-heptane	Prim./	D-5-Ether	2	− 5 (−10 − 0)	(1, 2)	LD_{50}-Rt-IP	60 (1)
		Adreno.	C-2-Barb	1.9	−14 −22	(16)	LD_{50}-Rt-SC	117 (59)
5[f]	2-Methylamino-heptane	Prim./	D-5-Ether	2	− 7 (−28 − 0)	(1, 2, 32)	LD_{50}-Rt-IP	60 (1)
6[f]	2-Methylamino-iso-octene	Prim./	D-5-Ether	2	− 10 (−14 − 0)	(1, 2)	LD_{50}-Rt-IP	70 (1)
							LD_{50}-Rb-SC	101 (57)
7	2-Amino-6-methyl-heptane	Adreno.	C-2-Barb.	1	−10 −16	(16)	...	...
8	2-Amino-4-methyl-hexane	Adreno.	C-2-Barb.	1.8	0 −8	16)	...	...

[a] Type of depressor response. Prim.—primary effect; Occas.—occasionally occurs depending on dose, etc.; Adreno.—occurs after an adrenergic blocking agent; Sec.—appears secondary to a primary pressor response.

[b] Species used: C—cat; D—dog; R—rabbit; H—human: Number of animals tested and anesthetic used: Ure.—urethane; Barb.—any barbiturate; Cbl.—chloretone; CH.—chloral hydrate; Dec.—decerebrate; Pith.—pithed.

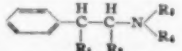
[c] Dose of a water-soluble salt, usually the hydrochloride, by intravenous injection unless otherwise stated. Range of dosages given in parentheses.

[d] Blood pressure response in mm. Hg except where stated as percentage change from preinjection level. Range of responses obtained given in parentheses.

[e] Type of acute toxicity test done, species used: Rt—rat; Rb—rabbit; M—mouse; GP—guinea pig; route of administration and dose of water-soluble salt in mg./Kg.

[f] A depressor effect which precedes the pressor response and which becomes more marked with each subsequent dose.

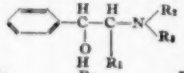
TABLE II.—PHENYL-ETHYLAMINE DERIVATIVES[a]



	R_1	R_2	R_3	R_4	Type	Animal	Dose, Kg./Mg.	Response, Mm. Hg	Ref.	Acute Toxicity	Mg./Kg.	Ref.
9	H	H	H	H	Adreno.	C-2-Barb.	1.3	−13	(16)	ALD-Rt-IP	100	(31)
10	H	H	H	CH_3	Occas.	C-5-Ure.	1.5 (1–5)	+20% (−35 + 54)	(54)	ALD-Rt-IP	180	(31)
11	H	H	H	C_2H_5	Occas.	D-?-Barb.	0.45 (0.1–0.95)	+3 (−30 + 32)	(34)	LD_{50}-M-IP	125	(34)
12	H	H	H	*iso*-C_3H_7	Prim.	D-?-Barb.	0.5	−30	(35)	LD_{50}-Rb-IV	75	(58)
13	H	H	H	*n*-C_3H_7	Prim.	D-?-Dec.	1.0	Depressor	(58)	LD_{50}-Rb-IV	58	(58)
14	H	H	CH_3	CH_3	Occas.	D-?-Barb.	0.54 (0.1–0.9)	+14 (−40 + 72)	(34)	LD_{50}-M-IP	135	(34)
15	H	H	C_2H_5	C_2H_5	Prim.	D-?-Barb.	0.65 (0.5–0.95)	−24 (−44 − 24)	(34)	...	...	...
16	H	CH_3	H	H	Adreno.	C-2-Barb.	2.94	−17	(16)	ALD-Rt-IP	23	(31)
					Occas.	C-5-Ure.	3.0 (3–5)	+16% (−29 + 36)	(54)	LD_{50}-M-SC	15–42	(60)
17	CH_3	H	H	H	Occas.	C-4-Ure.	3.0 (3–5)	−4% (−24 + 54)	(54)	ALD-Rt-IP	150	(31)
										LD_{50}-Rb-IV	55	(58)
18[b]	CH_3	H	H	CH_3	[b]	D-3-Ether	2.0 (1–3)	−40 (−25 − 50)	(3)	ALD-Rt-IP	200	(31)
					Adreno.	C-2-Barb.	2.4	−6 −32	(16)	LD_{50}-M-SC	400	(60)
19	C_2H_5	H	CH_3	CH_3	Prim.	D-?-Pith.	1.0	Depressor	(58)	LD_{50}-Rb-IV	53	(58)
20	C_2H_5	H	H	C_2H_5	Prim.	D-?-Pith.	1.0	Depressor	(58)	LD_{50}-Rb-IV	42	(58)
21	C_2H_5	H	C_2H_5	C_2H_5	Prim.	D-?-Pith.	1.0	Depressor	(58)	LD_{50}-Rb-IV	40	(58)
22[b]	CH_3	H	CH_3	Cinn.	Prim.	D-6-Barb.	5.0 (1–25)	−25 (−10 − 50)	(6)	LD_{50}-M-IV	20	(61)
23	H	C_6H_5	H	H	Prim.	R-?-?	3.4	−32	(26)	LD_{50}-M-IP	175	(26)

[a] Notation as in Table I.
[b] Following Paredrine Hydrobromide.

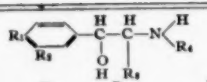
TABLE III.—PHENYL ETHANOLAMINE DERIVATIVES[a]



	R_1	R_2	R_3	Type	Animal	Dose/Kg.	Response, Mm. Hg	Ref.	Acute Toxicity	Mg./Kg.	Ref.
24	H	H	C_4H_9	Prim.	C-1-Pith.	4.0 mg.	−7	(14)	...	...	...
25	H	H	*iso*-C_3H_7	Prim.	D-?-Barb.	500 μg.	−36	(35)	LD_{50}-M-IP	180	(35)
26	C_6H_5	H	H	Occas.	C-1-Ure.	2.0 mg.	−1% (−9 + 7)	(54)	...	...	...
27	CH_3	H	H	Adreno.	C-2-Barb.	1.8 mg.	−14 (−10 − 18)	(16)	LD_{50}-Rt-SC	850	(59)
									LD_{50}-Rt-IV	85	(54)
28[b]	CH_3	H	CH_3	Adreno.	C-2-Barb.	1.2 mg.	−10 (−6 − 14)	(16)	LD_{50}-Rb-IV	65	(58)
29	CH_3	CH_3	C_2H_5	Occas.	D-?-Barb.	1.0 mg.	−10 +8	(9)	LD_{50}-Rb-IV	70	(61)
30	CH_3	H	Benzyl	Occas.	C-1-Pith.	5.0 mg.	Depressor	(14)	M. L. D.-Rb-IV	20	(14)
31	CH_3	H	*n*-C_3H_7	Prim.	C-1-Pith.	2.5 mg.	−6	(14)	M. L. D.-Rb-IV	50	(14)
32	CH_3	H	*n*-C_4H_9	Prim.	C-1-Pith.	2.0 mg.	−6	(14)	M. L. D.-Rb-IV	15	(14)
33	CH_3	H	*n*-C_5H_{11}	Prim.	C-1-Pith.	2.0 mg.	−12	(14)	M. L. D.-Rb-IV	20	(14)
34	CH_3	CH_3	Cinn.	Prim.	C-7-Barb.	1.0 mg.	−37	(45)	...	...	...
35	C_2H_5	H	H	Occas.	C-9-Ure.	5.0 mg. (1–20)	+9% (−41 + 15)	(54)	M. L. D.-Rb-IV	50	(14)
36	C_2H_5	C_4H_9	C_4H_9	Occas.	C-1-Pith.	500 μg.	−11 +15	(14)	M. L. D.-Rb-IV	50	(14)
37	*n*-C_3H_7	H	H	Prim.	C-3-Ure.	5.0 mg. (2–20)	−18% (−67 − 7)	(54)	...	...	...
38	*n*-C_3H_7	H	CH_3	Prim.	C-1-Pith.	2.0 mg.	−10	(14)	M. L. D.-Rb-IV	35	(14)
39	C_6H_5	H	H	Prim. ?	C-2-Ure.	15 mg. (10–20)	−81% (−100 − 61)	(54)	...	...	...
40	C_6H_{11}	H	H	Prim. ?	C-1-Ure.	10 mg.	−100%	(54)	...	...	...

[a] Notation as in Table I.
[b] Levo-ephedrine.

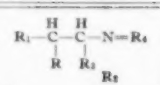
TABLE IV.—HYDROXYPHENYL-ETHANOLAMINE DERIVATIVES[a]



	R_1	R_2	R_3	R_4	Type	Animal	Dose/Kg.	Response, Mm. Hg	Ref.	Acute Toxicity	Mg./Kg.	Ref.
41	OH	H	H	CH_3	Adreno.	C-2-Barb.	700 μg.	0 −10	(16)	LD_{50}-M-IP	1000	(35)
42	OH	H	H	C_2H_5	Prim.	D-?-Barb.	500 μg.	−30	(35)	LD_{50}-M-IP	600	(35)
43	OH	H	H	*n*-C_3H_7	Prim.	D-?-Barb.	500 μg.	−19	(35)	LD_{50}-M-IP	300	(35)
44[a]	OH	H	H	*iso*-C_3H_7	Prim.	D-?-Barb.	200–500 μg.	−35 −63	(35)	LD_{50}-M-IP	370	(35)
45	H	OH	H	*iso*-C_3H_7	Prim.	D-?-Barb.	300 μg.	−30	(35)	LD_{50}-M-IP	320	(35)
46	OH	H	H	*n*-C_4H_9	Prim.	D-?-Barb.	500 μg.	−21	(35)	LD_{50}-M-IP	150	(35)
47	OH	H	H	*sec*-C_4H_9	Prim.	D-?-Barb.	250 μg.	−26	(35)	...	...	...
48	OH	H	H	*tert*-C_4H_9	Prim.	D-?-Barb.	250–500 μg.	−20 −60	(35)	LD_{50}-M-IP	250	(35)
49	OH	H	H	*iso*-C_4H_9	Prim.	D-?-Barb.	200 μg.	−4	(35)	LD_{50}-M-IP	220	(35)
50[a]	OH	OH	H	H	Adreno.	C-3-Barb.	20 μg.	−23	(4)	LD_{50}-M-IP	16	(17)
										LD_{50}-M-IV	10	(17)
51[a]	OH	OH	CH_3	H	Adreno.	C-3-Barb.	20 μg.	−30	(4)	*LD*-Rb-IV	1	(43)
					Adreno.	C-?-Ure.	...	Depressor	(43)	...	...	...
52[b] [a]	OH	OH	H	CH_3	Adreno.	C-3-Barb.	2 μg.	−45	(4)	LD_{50}-M-IP	8	(17)
										LD_{50}-M-IV	5	(17)
53[c] [a]	OH	OH	H	CH_3	Adreno.	C-?-Pith.	...	Depressor	(8)	LD_{50}-M-IP	5	(17)
					Adreno.	C-3-Barb.	2 μg.	−55	(4)	LD_{50}-M-IV	3	(17)
					Prim.	H-7	0.2 μg./min.	Dec. TPR	(21)	...	...	...
54	OH	OH	H	C_2H_5	Sec.	D-?-Barb.	1.7 μg.	−32	(38)	LD_{50}-M-IP	494	(38)
55[a]	OH	OH	CH_3	CH_3	Prim.	D-?-CH.	20–200 μg.	Depressor	(43)	*LD*-Rb-IV	1	(43)
					Prim.	D-8-Barb.	10 μg.	−25	(4)	...	...	...
					Prim.	C-6-Barb.	20 μg.	−20	(4)	...	...	...
					Adreno.	C-3-Barb.	2 μg.	−55	(4)	...	...	...
56[a]	OH	OH	C_2H_5	H	Prim.	C-10-Ure.	300 μg. (50–500)	−22% (−34 − 21)	(54) See also (12, 13)	LD_{50}-M-IV	117	(55)
57	OH	OH	C_2H_5	CH_3	Prim.	D-1-CH.	1–500 μg.	−5 −50	(43)	...	...	...
58	OH	OH	H	C_2H_5	Sec.	D-1-Barb.	2.3×10^{-8} mol.	−20	(40)	$LD_{50}/_{60}$-M-IP	10–15	(40)
59	OH	OH	H	*n*-C_3H_7	Sec.	D-1-Barb.	2.3×10^{-8} mol.	−13	(40)	$LD_{50}/_{60}$-M-IP	150/300	(40)
60[a]	OH	OH	H	*iso*-C_3H_7	Prim.	D-8-Barb.	10 μg.	−50	(4)	LD_{50}-M-IP	450	(37)
					Prim.	C-6-Barb.	20 μg.	−40	(4)	$LD_{50}/_{60}$-M-IP	120/300	(40)
					Adreno.	C-3-Barb.	2 μg.	−58	(4)	...	...	...
					Prim.	D-?-Barb.	0.6–1 μg.	−38 −41	(37)	...	...	...
					Prim.	D-1-Barb.	2.3×10^{-8} mol.	−50	(40)	...	...	...
61	OH	OH	H	*n*-C_4H_9	Prim.	D-?-Barb.	17 μg.	−45	(38)	LD_{50}-M-IP	347	(38)
62	OH	OH	H	*sec*-C_4H_9	Prim.	D-?-Barb.	1–2.4 μg.	−33 −37	(38)	LD_{50}-M-IP	480	(37)
					Prim.	D-1-Barb.	2.3×10^{-8} mol.	−32	(40)	$LD_{50}/_{60}$-M-IP	180/300	(40)
63	OH	OH	H	*tert*-C_4H_9	Prim.	D-?-Barb.	0.9 μg.	−43	(38)	LD_{50}-M-IP	370	(38)
					Prim.	D-1-Barb.	2.3×10^{-8} mol.	−60	(40)	$LD_{50}/_{60}$-M-IP	180/300	(40)

[a] Notation as in Table I. [b] Racemic epinephrine. [c] Levo-epinephrine.

TABLE V.—MISCELLANEOUS AMINES[a]



	R_1	R_2	R_3	R_4	Type	Animal	Dose/Kg.	Response, Mm. Hg	Ref.	Acute Toxicity	Mg./Kg.	Ref.
64	2-Hydroxyphenyl	OH	CH_3	H CH_3	...	C-leg	...	Dilator	(41)	...	...	...
65	2-Methoxyphenyl	CH_3	H	H_2	Prim.	D-?-Chl.	10 mg. total	−15	(22)	LD_{50}-Rt-IV	80	(22)
66	2-Methoxyphenyl	H	CH_3	H CH_3	Prim.	D-2-Barb.	5–25 mg.	−18 −24	(23)	LD_{50}-Rt-IV	50	(23)
										LD_{50}-Rb-IV	35	(23)
67	2-Methoxyphenyl	H	CH_3	di-CH_3	Prim. ?	D-?-Barb.	...	Depressor	(23)	LD_{50}-Rt-IV	40	(23)
68	2-Methoxyphenyl	H	CH_3	Benzyl	Prim. ?	D-?-Barb.	...	Depressor	(23)	LD_{50}-Rt-IV	20	(23)
69	4-Hydroxyphenyl	H	H	H_2	Adreno.	C-2-Barb.	0.9 mg.	−12 −14	(16)	M. L. D.-M-IP	800	(19)
					Adreno.	D-1-Barb.	0.5 mg.	−20	(5)	...	...	...
70	4-Hydroxyphenyl	H	H	di-CH_3	Occas.	C-8-Ure.	5 mg. (1–6)	+34% (−16 + 75)	(54)	...	...	...
71	4-Hydroxyphenyl	H	CH_3	H_2	Adreno.	C-2-Barb.	0.49 mg.	0 −8	(16)	LD_{50}GP-SC	175	(7)
72	4-Methoxyphenyl	OH	CH_3	H_2	Prim.	C-2-Ure.	7.5 mg. (5–10)	−15% (−20 − 10)	(54)	...	...	...
73	3,4-Dihydroxyphenyl	O	H	*iso*-C_3H_7	Occas.	D-?-Barb.	1.2 mg.	+21 to −28	(38)	LD_{50}-M-IP	470	(38)
74	2,6-Dihydroxyphenyl	CH_3	H	H_2	Prim.	D-1-Chl.	About 1 mg.	−16	(22)	LD_{50}-Rt-IV	15	(22)
75	2,6-Dimethoxyphenyl	CH_3	H	H_2	Prim.	D-1-Chl.	0.8	Depressor	(22)	LD_{50}-Rt-IV	90	(22)
76	3,5-Dimethoxyphenyl	CH_3	H	H_2	Prim.	D-1-Chl.	About 0.5 mg.	−22	(22)	LD_{50}-Rt-IV	70	(22)
77	2,4-Dimethoxyphenyl	OH	CH_3	H_2	Occas.	C-6-Ure.	7.5 mg. (1–10)	−8% (−50 + 11)	(54)	...	...	...
78	3,4,5-Trimethoxyphenyl	H	H	H_2	Occas.	C-5-Ure.	5 mg. (1–10)	+9% (−15 + 35)	(54)	...	...	...
79	3,4-Dimethoxyphenyl	H	H	H_2	Sec.	C-1-Pith.	4 mg. (SC)	Pressor then depressor	(19)	M. L. D.-M-IP	420	(19)
80	3-Methoxy-4-hydroxyphenyl	H	H	H_2	Sec.	C-?-Ether D-?-Barb.	10–20 mg.	Pressor then depressor	(27)	...	...	...
81	3-Methoxy-4-hydroxyphenyl	H	H	H_2	Prim.	C-?-Ether D-?-Barb.	2.5–10 mg.	−3 to −70	(27)	...	...	...
82	3-Methoxy-4-phenylmethoxyphenyl	H	CH_3	H_2	Prim.	CDR-?-?	...	Depressor	(25)	M. L. D.-M-IP	120	(25)
83	4-Methoxy-3-phenylmethoxyphenyl	H	CH_3	H_2	Prim.	CDR-?-?	...	Depressor	(25)	M. L. D.-M-IP	200	(25)
84	Cyclohexyl	H	H	di-CH_3	Prim.	D-?-Barb.	0.42 mg.	−7	(34)	LD_{50}-M-IP	85	(34)
85	Cyclohexyl	H	H	di-C_2H_5	Prim.	D-?-Barb.	0.66 mg.	−19	(34)	LD_{50}-M-IP	80	(34)
86	Cyclopentyl	H	H	di-CH_3	Prim.	D-?-Barb.	0.45 mg.	−21	(34)	LD_{50}-M-IP	85	(34)
87	Cyclopentyl	H	H	di-C_2H_5	Prim.	D-?-Barb.	0.78 mg.	−19	(34)	LD_{50}-M-IP	50	(34)

[a] Notation as in Table I.

Substitution of the entire benzyl group produces strong pressor agents. The pharmacology of the imidazolines is further complicated by the appearance of histaminic, cholinergic, and adrenergic blocking effects with certain structural changes.

DISCUSSION

The sympathomimetic agents are thought to bring their effects by acting on or through the adrenotropic (adrenergic) receptor mechanism of the effector cells. Classically, these receptors have been considered to be excitatory (*E*) or inhibitory (*I*), but recent evidence (4) has shown that these designations are not the best to explain all of the observed relationships. Certain excitatory effects are apparently related to inhibitory effects, and some inhibitory effects related to most of the excitatory responses. For this reason the two classes of adrenotropic receptors have been designed as *alpha* (vasoconstriction, uterine stimulation, intestinal inhibition, etc.), or *beta* (vasodilation, uterine inhibition, bronchial relaxation, and myocardial stimulation). According to this concept the depressor sympathomimetic agents act predominantly on the *beta* receptors.

Regardless of which receptor designation is used, certain relationships are apparent. The blood pressure effects of the sympathomimetic agents represent the balance between their constrictor, dilator, and myocardial actions. Epinephrine (No. 53), for example, acts on all types of adrenotropic receptors but the constrictor effect predominates so a pressor response usually occurs. Blocking the constrictor (*alpha* or *E*) receptors with an adrenergic blocking agent allows the dilator (*beta* or *I*) receptors to predominate and a depressor response occurs, i.e., epinephrine "reversal." Arterenol (No. 50) acts predominantly on the constrictor receptors so "reversal" after an adrenergic blocking agent occurs only when sufficiently large doses of this amine are administered. Isopropyl-Arterenol (No. 60) acts predominantly on the dilator receptors so its usual effect is depressor, except in rabbits which appear to have few dilator receptors. Methyl-epinephrine (No. 55) illustrates most strikingly this pressor-depressor balance; a small dose (5 μg./Kg.) produces a depressor response, a medium dose (100 μg./Kg.) gives a diphasic effect, while a large does (500 μg./Kg.) produces an almost pure pressor response (4, 43). Therefore, changes in chemical structure can produce depressor agents by: (*a*) producing or increasing dilator activity; or (*b*) decreasing constrictor activity to allow depressor effects to predominate.

Although too few comparative studies of the sympathomimetic depressor agents have been reported to warrant extensive theorization regarding the relationship of chemical structure to activity, certain general relationships can be pointed out.

The most fundamental of these is that depressor activity closely parallels pressor activity. For example, the N-isopropyl secondary amines show increasing depressor activity in the following order:

1. Phenyl-ethyl- (No. 12).
2. Phenyl-ethanol- (No. 25).
3. 3- or 4-hydroxyphenyl-ethanol- (No. 44 or No. 45).
4. 3,4-dihydroxyphenyl-ethanol- (No. 60).

The primary or N-methyl secondary amines show increasing pressor activity in exactly the same order. Furthermore, the effective doses of members of either the depressor or pressor series are in the same range. These facts constitute reasonably good evidence that both series (depressor or pressor) act upon physiologically similar systems.

Another observation is that increasing the length of the aliphatic portion of the basic phenethylamine structure often increases depressor activity. Compare, for example, the following compounds: No. 56 and No. 57 which have butyl side chains and which are normally depressor with No. 50 (Arterenol) and No. 52 (epinephrine) which have ethyl side chains and which are normally pressor; and compounds Nos. 35, 37, and 39 in which increasing side chain length increases depressor activity.

Other relationships are not so apparent but a few generalizations can be made. The tertiary amines are usually more depressor than primary amines, especially if one of the substituents is ethyl, as in Nos. 15, 21, 29, 85, 87, or cinnamyl, as in No. 22 and No. 34. Some of the tertiary amines also have nicotine-like actions as pointed out by Lands, *et al.* (34). Secondary amines are usually more depressor than primary amines, with the isopropyl or tertiary butyl amines being the most active.

No conclusions regarding the relationship of structure and depressor activity to acute toxicity can be drawn except for one limited series of amines, the 3,4-dihydroxyphenyl-ethanolamines. In this series the acute toxicity diminishes strikingly with increasing depressor activity with the exception of arterenol and epinephrine. Apparently in this series the acute toxicity is related chiefly to vasconstriction rather than to direct central nervous system effects.

At the present time the main therapeutic application of the depressor sympathomimetic amines is in the treatment of bronchial asthma. This is true because the bronchial dilator activity parallels the depressor activity. The following compounds which are primary depressor agents are available for clinical use: 3,4-dihydroxyphenyl-isopropyl-ethanolamine (isopropyl Arterenol, No. 60, Isuprel-Winthrop-Stearns, Norisodrine-Abbott), 3,4,-dihydroxyphenyl-butanolamine (ethyl norepinephrine, No. 56, Butanephrine-Winthrop-Stearns), and 2-methoxyphenyl-isopropyl-methylamine (No. 66, Orthoxine-Upjohn).

One of the imidazolines, benzyl-imidazoline (Priscoline-Ciba) is used as a vasodilator in the treatment of peripheral vascular disease. This use is presumably based on its adrenergic blocking action.

SUMMARY

The reported effects of 87 depressor sympathomimetic amines on arterial blood pressure and their acute toxicities have been presented. Although too few truly comparative studies have been reported to warrant extensive theorization regarding structure and activity certain general relationships have been pointed out.

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The Application of a Two-Phase System to the Volumetric Determination of Certain Medicinal Organic Compounds*

By RICHARD H. ROBINSON and ROBERT P. TANSEY

A procedure is described for the assay of various organic compounds containing an inorganic water-soluble portion. The method is simple to run and no special technique or apparatus is required. The assay results obtained check closely with other standard methods tried.

THE PROBLEM of finding a simple and efficient method for the assay of certain amine salts and salts of organic acids has presented a number of difficulties. In general, the proposed methods of ignition, extraction, or titration either require a considerable amount of technique, or they are limited in their application, or they make use of special reagents whose stability is uncertain. The ignition procedure whereby a salt is ignited to form sodium carbonate, or sodium sulfate, or sodium chloride is subject to many sources of error and is very time-consuming.

Hitchins (1) proposed an extraction procedure for the assay of certain water-soluble salts of organic acids now adopted by U. S. P. XIII as the official method for the assay of sodium salicylate (2). His method is very satisfactory but it is subject to some of the disadvantages inherent in extraction techniques. For certain compounds this method requires two extractions per assay. The titration method proposed by Green (3) using nonaqueous solutions meets the requirements of speed and accuracy but requires special reagents, variable procedures for different compounds, and is of limited application. In attempting to devise a procedure for the analysis of sodium phenobarbital, a method was developed that is applicable not only to this compound but can be applied with equal facility to certain other salts of organic compounds. The method is an improvement over the standard solvent extraction method.

EXPERIMENTAL

To about 25 ml. of recently boiled and cooled distilled water add about 500 mg. of a salt of an organic acid or amine salt to be assayed. To this solution add 20 Gm. of sodium chloride. If a halogen determination is being made use 40 Gm. of sodium nitrate in place of the sodium chloride. Then add 50 to 100 ml. of a solvent designed to dissolve the liberated organic acid. Titrate this mixture with either a standard solution of an acid or base depending on the substance being tested. The solution should be sufficiently agitated during titration. This may be accomplished either with the use of a mechanical stirrer or with vigorous shaking. Special care should be taken in mixing as the end point is approached, in order to be certain that all of the organic component is liberated from the aqueous portion and becomes dissolved in the organic solvent.

In titrating basic salts of an organic acid a 0.1 *N* sulfuric acid is added using bromcresol green as the indicator. Alkaloidal salts are titrated with 0.1 *N* sodium hydroxide using a meta cresol purple or phenolphthalein end point.

Table I shows some typical results obtained by using the procedure explained above. The sub-

TABLE I

Substance	Labeled Amount	Assay Results, Two-Phase System, %	Assay Results, Standard Extraction Method, %	Solvent Used
Phenobarbital Sodium	.....	100.2	100.0	Chloroform
Sodium Oleate	.....	98.9	99.2	Chloroform
Morphine Sulfate	.....	99.6	99.7	Chloroform or Ether
Atropine Sulfate	.....	100.0	100.0	Chloroform or Ether
Codeine Sulfate	.....	76.8[a]	76.5	Chloroform or Ether
d-Desoxyephedrine Hydrochloride	.....	99.8	99.6	Ether
Solution of Phenobarbital Sodium	2.2 gr./fl. oz.	100.0	98.2	Chloroform
Tablets *d*-Desoxyephedrine Hydrochloride	5 mg.	100.4	100.8	Ether
Tablets Morphine Sulfate	1/4 gr.	101.6	102.4	Chloroform
Capsules Pentobarbital Sodium	1 1/2 gr.	100.3	99.9	Chloroform
Tablets Quinine Sulfate	2 gr.	101.0	101.0	Chloroform

[a] Expressed as anhydrous codeine.

* Received December 23, 1949, from the Research Division of the Maltbie Laboratories, Inc., Newark, N. J.

stances analyzed included both raw materials used in our manufacturing plant and some of our finished products.

DISCUSSION

The data presented indicate that a close correlation is obtained between the standard extraction methods of assay and the proposed two-phase titration procedure. The latter method has the advantage of not being limited in its application as are the various extraction, ignition, and titration procedures. It meets the requirements of speed and accuracy and no special reagents are necessary. One basic procedure can be used for the various medicinal organic compounds being tested.

SUMMARY

1. A method is presented for analyzing salts of organic acids and amine salts by titrating their inorganic water-soluble portion, while simultaneously removing the organic portion.

2. Data to substantiate the accuracy and usefulness of the method are recorded.

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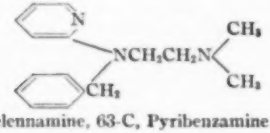
The Spectrophotometric Determination of Methapyrilene Hydrochloride and Related Antihistaminic Derivatives of N-(2-pyridyl)-ethylenediamine*

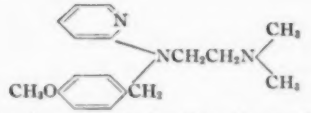
By ERIC W. MARTIN and JOSEPH W. E. HARRISSON

A rapid spectrophotometric assay method for the determination of methapyrilene and structurally related antihistaminic compounds in pharmaceutical preparations is described. Absorption spectra and a standard curve are presented with typical analytical results which indicate that the method is exceptionally accurate as well as general in its application.

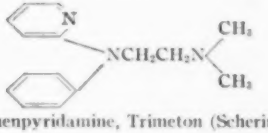
METHAPYRILENE (Thenylpyramine) hydrochloride is one of a series of antihistaminic compounds, containing the N-(2-pyridyl)-ethylenediamine skeleton, which are sold under a rapidly increasing number of trade names. The formulas for the pyridyl derivatives most widely recognized at the present time (1, 3, 4, 6) have been grouped to indicate the similarities in their structural formulas. Only the bases are shown. However, all of them are marketed as crystalline salts, such as the hydrochloride, maleate, and citrate.



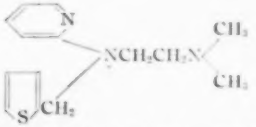
Tripelennamine, 63-C, Pyribenzamine (Ciba)



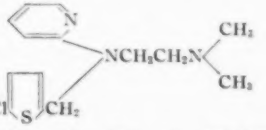
Pyranisamine, 2786 R. P., Neoantergan (Merck)



Prophenpyridamine, Trimeton (Schering)

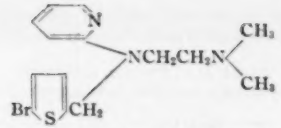


Methapyrilene, AH-42, Thenylpyramine (Monsanto), Thenylene (Abbott), Histadyl (Lilly), Pentryl (Maltine), Semikon (Massengill)



Chlorothenylpyramine, Chlorothen (Whittier), Tagathen (Lederle)

* Received January 6, 1950, from LaWall and Harrisson, Philadelphia, Pa.



Bromothenylpyramine, Bromothen (Lederle)

The compounds for which structural formulas are given were found to possess characteristic absorption spectra (3) with well-defined maxima. Since the ultraviolet light at the band maxima appears to be absorbed for each substance in perfect agreement with Beer's law, a standard curve can be constructed by plotting absorbancy at the proper wave length against concentration of the antihistaminic compound. The curves so constructed can be employed for the routine control analysis of pharmaceutical preparations containing these substances, provided interferences when present are eliminated by the use of suitable blanks. For example, placebo tablets are often used for the preparation of a reference solution for each tablet formulation, and the correction which is determined for tablets with a given lot number can be used for all tablets in that lot. The placebo tablets should contain all the ingredients of the same lot number as the tablet being assayed but none of the antihistaminic agent. The same method of treatment is customarily employed for elixirs, parenteral solutions, ointments, etc. Occasionally the blank is so small that its determination is unnecessary.

EXPERIMENTAL

Materials.—Monsanto Thenylpyramine hydrochloride (medicinal grade) was recrystallized twice from anhydrous ethanol and dried at 100° for four hours. All other chemicals and solvents were of reagent grade.

Determination of Spectra.—The absorption spectra reported in this paper were determined in 1-cm. calibrated, matched silica cells with a Beckman Model DU Quartz Spectrophotometer equipped with a hydrogen lamp and an ultraviolet accessory set. The hydrogen lamp was used throughout the spectral region reported, without the use of a filter. The solvents were used as the reference liquids. The solutions employed for the determination of the absorption spectra were prepared by dissolving 100.0 mg. of the purified salt in 500 ml. of solvent and diluting a 10-ml. aliquot (2 mg. of salt) to 100 ml. with the same solvent. Table I summarizes the *p*H values for solutions containing 2 mg./100 ml. of the solvent, and tabulates the wave lengths at which maximum absorption occurred and the $E^{1\%}_{1\ cm.}$ values at these maxima.

The above values indicate that there is a decided shift in the spectrum of Thenylpyramine hydrochloride with a change in *p*H. The shift is practically negligible at the 237–238 mμ band, but is considerable in the region 304–314 mμ. Examination of the *E* values shows only a slight change in the amount of absorption with *p*H in the region 237–238 mμ, but a large variation is noted in the region 304–314 mμ. From the above data it is concluded that most satisfactory results can be obtained by constructing a standard curve (absorbancy vs. concentration) at $\lambda_{237.5}$ at *p*H 2 or less.

TABLE I.—THENYLPYRAMINE HYDROCHLORIDE SOLUTIONS

Solvent	*p*H	$\lambda_{max.}$	$E^{1\%}_{1\ cm.}$
10° *N* HCl	0.5	237.5	608
		314	273
10^{-2} *N* HCl	2.1	237.5	608
		314	271
10^{-4} *N* HCl	4.7	238	590
		304	162
Water	6.3	238	590
		304	157

The absorption spectra at *p*H 2.1 and *p*H 4.7 are shown in Fig. 1. The concentration of Thenylpyramine hydrochloride represented by these curves is 2 mg./100 ml. Curve A is the spectrum obtained when 10^{-2} *N* HCl is the solvent and curve B is the spectrum in 10^{-4} *N* HCl. At $\lambda_{237.5}$ the absorbancy differs by only about 1% whereas the difference between the absorbancy at λ_{304} and at λ_{314} is about 57%.

The Standard Curve.—Three solutions of Thenylpyramine Hydrochloride were prepared, containing 0.5 mg., 1.0 mg., and 2 mg., respectively, in 100 ml. of 1.0 *N* HCl. The absorbancies of these solutions at $\lambda_{237.5}$ were plotted against concentration, expressed in mg./100 ml. of solution. The curve so obtained was a straight line, indicating that Beer's law is followed very closely. It is shown in Fig. 2.

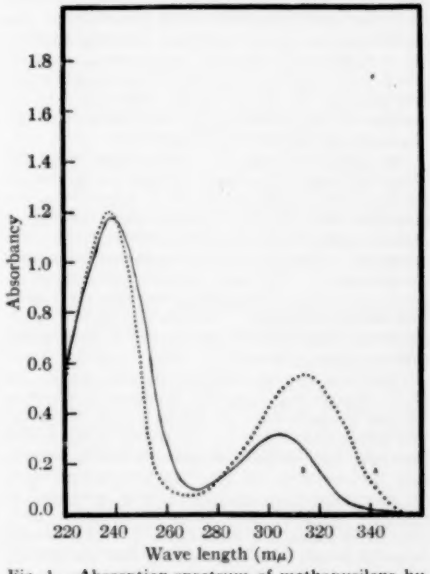


Fig. 1.—Absorption spectrum of methapyrilene hydrochloride. *A*, *p*H 2.1; *B*, *p*H 4.7.

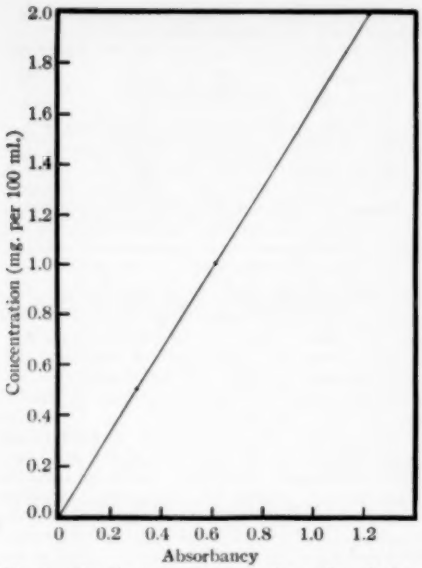


Fig. 2.—Standard curve for methapyrilene hydrochloride.

DISCUSSION

When absorption maxima are constant and intense, as for example, that recorded in Fig. 1 at $\lambda_{337.4}$ for Thenylpyramine hydrochloride, the spectrophotometric method of assay is highly accurate and many interferences are minimized to the extent that they either fall within the range of experimental error, or are eliminated. The convenience and rapidity of this type of analytical procedure, resulting in economy of both time and material, have resulted in its adoption as a routine procedure whenever possible. It can be applied, apparently, to the analysis of all antihistaminic compounds with the N-(2-pyridyl)-ethylenediamine group.

The direct utilization of light absorption in the ultraviolet region is usually superior to procedures requiring the development of a color with the attendant sources of experimental error, although it may be desirable for special purposes to adopt a colorimetric procedure for estimating antihistaminic compounds (5). The spectrophotometric method reported above, however, is more sensitive than many colorimetric procedures. As little as 4 μg. of Thenylpyramine hydrochloride per ml. can be determined with an accuracy of ±0.5%.

Typical analytical results obtained when this spectrophotometric procedure is applied to pharmaceutical preparations are tabulated in Table II. The preparations were all accurately compounded from pure Thenylpyramine hydrochloride in order to test the accuracy of the method in mixtures. Placebo preparations, suitably diluted, were used as reference liquids. The greatest deviation from theoretical recorded is less than 2%. The average deviation is only 0.66%, indicating that the method is highly accurate. Throughout the development of the procedure and the assay of the preparations, a calibration curve was used for the silica cells. Therefore all absorbancies reported are corrected.

TABLE II.—TYPICAL ANALYSES

Preparation	Concentration of Thenylpyramine HCl—Found	Theoretical	Percentage Deviation from Theory
Elixir	50.17 mg./ml.	50 mg./ml.	0.34
Elixir	49.83 mg./ml.	50 mg./ml.	−0.34
Syrup	49.97 mg./ml.	50 mg./ml.	−0.16
Syrup	49.84 mg./ml.	50 mg./ml.	−0.32
Solution	19.70 mg./100ml.	20 mg./100ml.	−1.48
Solution	19.61 mg./100 ml.	20 mg./100 ml.	−1.97

The stability of Thenylpyramine hydrochloride in the presence of acid at elevated temperatures was tested during the development of the above method. The absorbancy and *p*H were determined for two solutions containing 2 mg. of the salt per 100 ml. of solution. The solutions were autoclaved at 15 lb. for twenty minutes, cooled to room temperature, and the *p*H and absorbancies were again determined. The results are recorded in Table III.

TABLE III.—STABILITY OF THENYLPYRAMINE TO HEAT AND ACID

Solvent	*p*H Before Autoclaving	*p*H After Autoclaving	Absorbancy Before Autoclaving	Absorbancy After Autoclaving
10^{-2} *N* HCl	2.1	2.1	1.205	1.205
Water	6.3	6.3	1.179	1.179

Thenylpyramine hydrochloride was found to withstand heating in the autoclave when dissolved either in water or in dilute acid. This was indicated by the fact that neither the *p*H nor the absorbancy of the solutions was altered by the treatment.

SUMMARY

1. A highly accurate, rapid, and convenient spectrophotometric assay procedure for the determination of Thenylpyramine hydrochloride in pharmaceutical preparations is described. This method appears to be general for N-(2-pyridyl)-ethylenediamine compounds.

2. The absorption spectra of the salt in solutions of various *p*H values are shown. A shift in the absorption spectrum of considerable magnitude occurred at one of the maxima when the *p*H was decreased from 4 to 2 whereas the other maximum remained constant.

3. The salt is stable. It withstands autoclaving at 15 lb. pressure for twenty minutes in both acid and aqueous solutions.

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Arylaminoheterocycles. V. Anilino-*s*-triazines*

By DONALD F. WALKER, YVON J. L'ITALIEN, WILLIAM M. PEARLMAN, and C. K. BANKS

The preparation of a number of new triamino-*s*-triazines is reported.

THE interesting physiological activities exhibited by *s*-triazines (1–4) suggested that substituted triamino-*s*-triazines would be worthy of investigation. As one set of variants of this structure, the monoanilino-diamino-*s*-triazines were suggested by the previously investigated anilinopyrimidines (5). 2-Anilino-4,6-diamino-*s*-triazine was prepared by Klason by heating 2-chloro-4,6-diamino-*s*-triazine in an excess of aniline at 150° (6). This method, however, is not generally applicable for many of the substituted derivatives and the yields are frequently low. In addition, the problems of purification are numerous.

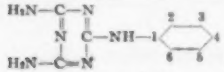
In a previous study (7) it was demonstrated that the reaction of 2-chloro-4,6-diamino-*s*-triazine and aniline was quantitative in dilute aqueous solutions when a trace of acid was added as a catalyst. From this and other work it was generalized that this mechanism of nucleophilic attack of arylamines upon certain halogen-substituted nitrogen-containing heterocycles was effective on those halogens substituted on a carbon adjacent to or twice removed from the ring nitrogen. These halogens are naturally activated by a tautomeric electron displacement from the double bond to nitrogen resulting in a small positive charge, $\delta_T{}^+$, at the α and γ positions. The intensification of activity by acid was postulated to result from a proton combining with the ring nitrogen to produce a formal positive charge on the nitrogen and an inductive effect ($-I$), on the ring. Since the effects are additive, the charge at the α and γ positions becomes $\delta_T{}^+ + \delta_I{}^+$ and these positions become more vulnerable to nucleophilic attack. The reactions of halogenated pyrimidines, thiozoles, pyridines, benzothiazoles, benzoquinolines, isoquinolines, and quinolines with aromatic amines have also been studied (1, 5, 8–13) and the mechanism postulated above appears to be generally applicable to the reaction of halogenated heterocyclic compounds with arylamines. Of course the nature of the amine and the tautomeric differences of the ring systems have some influence upon the course of the reactions. Since numerous monochlorotriazines (14) were available, they were reacted under standard conditions with appropriate arylamines, varying only the reaction time. While this would not allow for a rigorous evaluation of reaction rates due to the varying solubilities of the reactants, it was sufficient to indicate some gross differences due to structural variation. In most instances, however, the reactants formed a suspension due to the low solubilities in water.

It has been noted by Curd, *et al.* (10), and by ourselves (7) that dialkylaminoalkylamines failed to condense with this type of haloheterocycle under similar conditions. If the theory advanced for the reaction mechanism is correct, the reaction involves the free amine with its unshared electron pair rather than the "ammonium" type ion. Thus, while acid activates the halogen atom of the heterocycle, it tends to decrease the available free amine by withdrawal as the "ammonium" ion. This is not serious with aryl amines which exist principally as the free amine in solution at *p*H values above *p*H 1 and are converted to the ion in strong acid only. Alkyl amines, however, are converted to a large extent to the ionic form in acid solution, removing a large amount of the amine from possible reaction. Previously it had been noted that morpholine reacted with 2-amino-4-chloropyrimidine (5), which is in keeping with the theory since it has a low basic strength. Similarly, in a buffered solution which was only slightly acid, dimethylamine would condense (14). Further exploration indicated that mixed alkyl-aryl amines (N-methyl; ethyl- and benzylaniline) and piperidine behaved similarly.

Purification of the products frequently presented a problem since recrystallization from the usual solvents failed to remove unreacted starting materials. A purification was finally achieved by condensing in the presence of an excess of the amine and then removing the excess amine by steam distillation. Another difficulty encountered was the innate stability of the triazine ring, this chemical perversity frequently resulted in irregular analytical results. Kjeldahl nitrogen determinations were frequently low by as much as the equivalent of one of the nitrogens present. Dumas nitrogen values were not reproducible and

* Received February 6, 1950, from the Research Laboratories of Parke, Davis and Company, Detroit, Mich.

TABLE I.—2,4-DIAMINO-6-RNH-*s*-TRIAZINES



	M. P., °C.	Yield, %	Recrystallized from	Analyses, %[a] C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
Unsubstituted (6)	284–286	82	Ethanol	...	...	...	...	41.6	41.4
2-CH_3	211–212	81	Ethanol-Water	...	...	...	...	38.9	39.2
3-CH_3	229–230	82	Ethanol-Water	...	...	...	...	38.9	38.6
4-CH_3	265–266	80	Ethanol-Water	55.54	55.76	5.59	5.30	...	...
2,4-$(CH_3)_2$	239–241	92	Ethanol-Water	...	...	...	...	36.5	36.3
2,5-$(CH_3)_2$	237–239	89	Ethanol-Water	...	...	...	...	36.5	36.2
2-Cl	205–208	81	Acid-Base	45.67	45.87	3.83	3.80	...	...
3-Cl	173–174	98	Ethanol-Water	45.67	45.60	3.83	3.74	...	...
4-Cl	245–249	82	Acid-Base	45.67	45.40	3.83	3.81	...	...
2,4-$(Cl)_2$	255–257	86	Acid-Base	...	...	...	...	31.0	30.9
2,5-$(Cl)_2$	228–230	84	Acid-Base	39.87	40.22	2.97	2.89	...	...
3,4-$(Cl)_2$	210–211	98	Ethanol-Water	...	...	...	...	31.0	30.8
2-OH	257–259	87	Ethanol	49.54	49.47	4.58	4.50	...	...
3-OH	241–242	76	Methanol	49.54	49.87	4.58	4.62	...	...
4-OH	282–283	88	Methanol	49.54	49.66	4.58	4.73	...	...
2-OC_2H_5	203–205	76	Ethanol	...	...	...	...	34.1	34.0
3-OC_2H_5	211–212	82	Ethanol	53.65	53.98	5.69	6.20	34.1	33.9
2-NO_2	300	..	Ethanol	43.72	43.42	3.66	3.50	...	...
3-NO_2	144–145	99	Dioxane	43.72	44.03	3.66	3.71	39.7	39.5
4-NO_2	300	..		43.72	44.04	3.66	3.59	...	...
2-COOH	Indefinite	42	Acid-Base	48.78	48.48	4.09	4.14	...	...
3-COOH	304–306	42	Acid-Base	...	...	...	...	34.2	34.4
4-COOH	300	68	Acid-Base	48.78	48.44	4.09	4.07	34.2	34.1
4-$COCH_3$	...	98	Acid-Base	...	...	...	...	34.4	34.4

[a] The analyses were performed by the Microanalytical Laboratory of our Research Department under the direction of A. W. Spang and C. E. Childs.

those which were within an acceptable range with respect to precision and amount were obtained only after extremely high combustion temperatures were used. Addition of copper acetate and lead peroxide failed to improve the assay procedure. Some compounds which could not be assayed for nitrogen were analyzed for carbon and hydrogen. Here again, some of the compounds failed to burn properly and reproducible values were not obtained. The propensity of triazines to form cyanogen compounds which may react with copper oxide to give undecomposable salts was frequently noted in the combustion tubes. A group of the triazines was left for which no analytical proof of structure could be obtained. Examination of the structural nature of these compounds sheds no light as to why these compounds should differ from those for which acceptable analyses were obtained. On the other hand, even those compounds for which reportable assays were obtained indicated, by the analytical difficulties encountered and the rather large limits of precision which were observed, that this class of compounds in general may be said to be extremely difficult to analyze by extant procedures.

The compounds have been studied against a number of infectious organisms and have been tested for several pharmacodynamic properties. A large number of activities of a low order were noted but no activity of sufficient magnitude to be pharmaceutically useful was noted.

EXPERIMENTAL

The 2-R_1R_2N-4-R_3R_4N-6-chloro-*s*-triazine (0.1 mole) and the amine (0.11 mole) were suspended in a convenient volume of water, generally one liter, 1 ml. of concentrated hydrochloric acid added, and the suspension refluxed until complete reaction occurred. This would be judged by the change in character of insolubles, by solution of insolubles or in a few instances where all the reactants were soluble, by following the disappearance of the aryl amine by a diazonium test. The resulting solution or suspension was then made neutral if no acidic groups were present in the molecule, otherwise the isoprecipitation point was determined on a small sample and applied to the entire run. All of the triazinylamines were insoluble and were filtered off. In those instances in which the starting amine was steam volatile, any unreacted amine was removed with steam and the residue then recrystallized from appropriate solvents. Where good recrystallization solvents could not be found, solution in acid or alkali and subsequent slow precipitation was used to purify the crude products.

TABLE II.—MISCELLANEOUS TRIAMINO-*s*-TRIAZINES

2	4	6	M. P., °C.	Yield, %	Recrystallized from	Analyses, % C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
$-NH_2$	$-NH_2$	Naphthylamino	>300	75	Benzene	61.88	62.18	4.79	4.88	...	...
$-NH_2$	$-NH_2$	N-Methylanilino	257–259	97	Ethanol-Water	...	...	...	...	38.9	38.9
$-NH_2$	$-NH_2$	N-Ethylanilino	215–217	94	Ethanol-Water	...	...	...	...	36.5	36.5
$-NH_2$	$-NH_2$	N-Benzylanilino	311–314	99	Ethanol-Water	65.73	65.57	5.50	5.40	...	...
$-NH_2$	$-NH_2$	Piperidino	216–217	22	Water	...	...	...	...	43.3	43.5
$-NH_2$	$-NH_2$	Morpholino	236–240	48	Water	...	...	...	...	42.9	43.0
$-NH_2$	$-NHCH_3$	Anilino	84–86	48	Water	55.52	55.25	5.60	5.59	...	...
$-NH_2$	$-NHC_2H_5$	Anilino	153–155	49	Water	57.37	57.41	6.13	5.81	...	...
$-NH_2$	$-NHCH_2C(CH_3)=CH_2$	*p*-Chloroanilino·HCl	237–239	64	Ethanol	47.71	47.67	4.90	5.20	...	...
$-NH_2$	$-NHCH_2C(CH_3)=CH_2$	*p*-Toluidino	137–139	60	Ethanol	62.11	62.05	6.71	6.66	...	...
$-NH_2$	$-NHC_2H_4OH$	Anilino	156–158	76	Water	...	...	...	...	34.2	34.1
$-NH_2$	$-NHC_2H_4OH$	*m*-Chloroanilino	147–150	43	Water	47.06	46.83	4.67	4.70	...	...
$-NH_2$	$-NHC_2H_4OH$	*p*-Chloroanilino	173–174	40	Water	47.06	46.86	4.67	4.48	...	...
$-NH_2$	$-NHCH_2CHOHCH_3$	Anilino	138–140	..	Water	...	...	...	...	32.3	32.2
$-NH_2$	$-N(CH_3)_2$	*o*-Chloroanilino	133–135	50	Ethanol-Water	49.90	49.70	4.94	4.90	...	...
$-NH_2$	$-N(CH_3)_2$	*p*-Chloroanilino	173–175	50	Ethanol-Water	49.90	50.13	4.94	4.96	...	...
$-NH_2$	$-N(CH_2CH=CH_2)_2$	*p*-Chloroanilino	137–141	..	Ethanol-Water	56.86	56.62	5.41	5.26	...	...
$-NH_2$	$-N(CH_2CH=CH_2)_2$	*p*-Toluidino	119–121	60	Ethanol	64.83	65.01	6.80	6.61	28.4	28.3
$-NH_2$	$-N[CH_2C(CH_3)=CH_2]_2$	*o*-Chloroanilino	78–81	44	Ethanol-Water	59.20	59.52	6.13	6.09	...	...
$-NH_2$	$-N[CH_2C(CH_3)=CH_2]_2$	*p*-Chloroanilino	154–157	..	Ethanol-Water	59.20	59.35	6.13	6.30	...	...
$-NHCH_3$	$-NHCH_3$	2,5-Dichloroanilino	153–155	76	Ethanol-Water	44.16	44.11	4.05	4.08	...	...
$-NHC_2H_5$	$-NHC_2H_5$	Anilino·2HCl	178–180	70	Ethanol·HCl	47.13	47.38	6.09	6.01	...	...
$-NHC_2H_5$	$-NHC_2H_5$	*m*-Chloroanilino·HCl	165–167	94	Ethanol·HCl	47.44	46.98	5.51	5.35	...	...
$-NHCH_2CH=CH_2$	$-NHCH_2CH\ CH_2$	*o*-Chloroanilino	56–59	..	Ethanol-Water	57.04	57.08	5.42	5.88	...	...
$-NHCH_2CH=CH_2$	$-NHCH_2CH\ CH_2$	*p*-Chloroanilino	103–106	66	Ethanol-Water	57.04	56.82	5.42	5.33	26.6	26.7
$-NHC_2H_4OH$	$-NHC_2H_4OH$	Anilino	130–132	..	Water	...	...	...	...	29.0	28.9
$-NHCH_2CHOHCH_3$	$-NHCH_2CHOHCH_3$	Anilino·HCl	150–152	..	Dioxane-Benzene	...	...	...	...	23.7	23.6
$-NHCH_2COOH$	$-NHCH_2COOH$	Anilino·$3H_2O$	...	..	Acid-Base	...	...	...	...	22.5	22.4
$-N(CH_3)_2$	$-N(CH_3)_2$	*o*-Chloroanilino	114–117	43	Ethanol-Water	53.33	53.66	5.85	5.99	...	...
$-N(C_2H_5)_2$	$-N(C_2H_5)_2$	Anilino	87–89	90	Dioxane-Water	64.93	65.33	8.34	8.15	...	...
$-N(C_2H_5)_2$	$-N(C_2H_5)_2$	*p*-Chloroanilino	134–135	76	Ethanol-Water	58.52	58.50	7.22	7.07	...	...
$-NH_2$	$-N(C_6H_5)C_2H_4OH$	$-N(C_6H_5)\ C_2H_4OH$	158–159	10	Ethanol	62.96	62.87	6.12	5.90	...	...
$-NH_2$	$-Cl$	4-Carboxy-3-hydroxyanilino	263–266	54	Acid-Base	42.64	42.72	2.86	2.76	...	...

SUMMARY

A number of new triamino-*s*-triazines were prepared.

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Alpha-Chloro-Delta-Aryloxyvaleric Acids*

By DONALD F. WALKER†

A method for the decarboxylation of certain substituted chloromalonic acids is described. A number of new aryloxypropylmalonic and aryloxypropylchloromalonic acids have been prepared and are reported. In antifungal screening tests the two valeric acids exhibited slight activity which was insufficient to warrant further investigation.

THE interesting preliminary antifungal tests obtained with α-chloro-δ-phenoxyvaleric acid in our laboratories prompted a study of variations in the aryl radical of related products. Subsequent to the inception of this work, Weaver and Whaley (1) reported the fungicidal activity of α-bromoacetamides and postulated that this activity may be attributed to the α-halogen atom.

The preparation of these compounds followed a well-known synthetic route. The aryloxyalkyl halides of Table I were prepared by the method of Marvel and Tanenbaum (2). Some difficulty was experienced in attaining analytical purity but the materials were satisfactory for later reactions. The aryloxyalkyl halides were reacted with ethyl malonate by the method of Merchant, Wickert, and Marvel (3). The crude esters, not being distillable at the highest vacuum available in our laboratory, were hydrolyzed at room temperature by the method of Cheney and Piening (4). Modifications of the procedure at this step made it possible to eliminate impurities carried along from previous reactions. As reported by Cheney and Piening (4), sulfuryl chloride gave yields of the α-chloroaryloxypropylmalonic acids which were a substantial improvement over those obtained by direct halogenation. Decarboxylation of the aryloxypropylchloromalonic acid presented the greatest problem encountered in the series. Uncrystallizable gums and undistillable oils often resulted so that many of the final products desired were not obtained.

Results of the antifungal testing of these compounds will be reported at a later time.

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† The author wishes to acknowledge the assistance and encouragement of C. K. Banks and to thank A. W. Spang and Charles E. Childs for microanalyses performed.

EXPERIMENTAL

Aryloxypropyl Bromides.—Following the procedure of Marvel and Tanenbaum (2) for the preparation of phenoxypropyl bromide, a mixture of the appropriate phenol (0.3 mole) and trimethylene bromide (0.5 mole) in water was heated to boiling under reflux with mechanical stirring. A dilute solution of NaOH (0.4 mole) in 75 ml. of water was added dropwise over a period of thirty minutes. Refluxing with vigorous stirring was continued for twenty-four hours. The excess alkylhalide was removed by steam distillation. The alkaline residue was extracted several times with ether. The ether extracts were combined and in turn extracted with dilute alkali. The ether layer was dried and the solvent removed by distillation. The residual oil was distilled *in vacuo* to obtain the desired aryloxypropyl bromide.

4-Chloro-2-isopropyl-5-methylphenoxyethoxyethyl Chloride.—Reaction of the requisite sodium phenate with β,β′-dichloroethyl ether as in the above procedure gave a 43% yield, b. p. 132–138° at 0.2 mm.

Anal. Calcd. for $C_{14}H_{20}Cl_2O_2$: C, 57.74; H, 6.92. Found: C, 58.04; H, 6.93.

α-Methyl-β-[β-(4-chloro-2-isopropyl-5-methylphenoxy)-β-methylethoxy]-ethyl Chloride.—Reaction of the requisite sodium phenate with β,β′-

TABLE I.—ARYLOXYALKYL GROUP

Compound	Bromide[a] Yield, %	Bromide[a] B. P., °C.[b]	Ethyl Malonates[c] Yield, %
β-Naphthoxypropyl-	40	56–57[d]	85
2,4-Dichlorophenoxypropyl-	66	170–174/12 mm.[e]	82
2,4,5-Trichlorophenoxypropyl-	62	192–193/9 mm.	82
4-Chloro-2-isopropyl-5-methylphenoxypropyl-	25	154–157/5 mm.	82
4-Chloro-2-isopropyl-5-methylphenoxybutyl-	79	[f]	69

[a] Identity established in subsequent reactions. [b] Uncorrected. [c] Unable to distill. [d] Melting point, recrystallized from ethanol. [e] Prepared as an intermediate, Newman, M. S., Fones, W. S., and Renoll, M. W., *J. Am. Chem. Soc.*, **69**, 718(1947). Chloride prepared as an intermediate, Synerholm, M. E., and Zimmerman, P. W., *Contribs. Boyce Thompson Inst.*, **14**, 369(1947). [f] Crude used in next reaction.

dichloroisopropyl ether as in the preceding procedure gave an 8% yield, b. p. 132–133° at 0.3 mm.

Anal. Calcd. for $C_{14}H_{20}Cl_2O_2$: C, 60.19; H, 7.58. Found: C, 60.31; H, 7.40.

Ethyl γ-aryloxypropylmalonates (3).—Sodium ethoxide was prepared in absolute ethanol in the usual manner. Freshly distilled malonic ester (0.25 mole) was added slowly with mechanical stirring to the solution of sodium ethoxide (0.5 mole). The mixture was stirred and heated to reflux while a solution of aryloxypropyl bromide (0.2 mole) in 150 ml. absolute ethanol was added dropwise over a period of one hour. Stirring and refluxing were continued for four hours. Ethanol was then removed by distillation. The reaction mixture was cooled, suspended in water, and extracted with several portions of ether. The ether extracts were combined and dried over anhydrous sodium sulfate. The ether was removed and all constituents volatile at a bath temperature of 200° in a vacuum of 12 mm. were distilled off. A sufficiently high vacuum to effect distillation of the crude esters could not be achieved. The crude esters were used in the hydrolysis.

Aryloxypropylmalonic Acids (4).—A solution of the aryloxypropyl malonic ester (0.25 mole) in 20 ml. of ethanol was hydrolyzed by adding dropwise a solution of KOH (1.2 moles) in 300 ml. of water with stirring and cooling in an ice bath. The mixture was mechanically stirred at room temperature for twenty-four hours to obtain a pale yellow to amber colored solution. The alkaline solution was extracted with several portions of ether to remove alkali-insoluble unreacted materials, charcoaled, and filtered. The filtrate was cooled in an ice bath to 0–5° and slowly acidified with a dilute solution of HCl until acid to congo red. The semisolid precipitate was extracted with ether and the ether extracts combined and dried. The ether was removed in a stream of air and the residual gummy solid was treated with 200 ml. of low boiling petroleum ether to obtain a light tan solid material which could be filtered off. The solid malonic acids were dried *in vacuo* at room temperature (see Table II).

Aryloxypropylchloromalonic Acids (4).—Sulfuryl chloride (0.1 mole) was added slowly to a mechanically stirred solution of the aryloxypropylmalonic acid (0.1 mole) in 200 ml. of anhydrous ethyl ether. The reaction mixture was stirred for one hour at room temperature and heated to reflux for one hour. The reaction mixture was cooled and the ether removed in a stream of air at room temperature. The residual oil was suspended in 100 ml. of low-boiling petroleum ether, whereupon it solidified slowly. The desired aryloxypropylchloromalonic acids were filtered off and dried *in vacuo* over KOH at room temperature. The yields were nearly quantitative (see Table III).

α-Chloro-δ-(β-naphthoxy)valeric Acid.—Ten grams of γ-(β-naphthoxy)-propylchloromalonic acid was decarboxylated by heating in a suitable bath at 170° until all evolution of gas had ceased and for five minutes longer. A dark oil which solidified on cooling was obtained in 87% yield.

Recrystallization from carbon tetrachloride gave a light tan solid, m. p. 125–217° (uncorr.).

Anal. Calcd. for $C_{15}H_{19}ClO_3$: C, 64.63; H, 5.42; Neutral Eq., 278.7. Found: C, 64.73; H, 5.28; Neutral Eq., 278.0.

TABLE II.—ARYLOXYPROPYLMALONIC ACID[a]

	Formula	Yield, %	M. P., °C.[b]	Neutral Equivalent Found	Neutral Equivalent Calcd.
γ-(β-Naphthoxy)propylmalonic acid	$C_{16}H_{16}O_5$	62	168–170, dec.	145.8	144.2
γ-(2,4-Dichlorophenoxy)propylmalonic acid[c]	$C_{12}H_{12}Cl_2O_5$	60	120, dec.	152.6	153.6
γ-(2,4,5-Trichlorophenoxy)propylmalonic acid	$C_{12}H_{11}Cl_3O_5$	70	145–147	171.4	170.8
γ-(4-Chloro-2-isopropyl-5-methylphenoxy)propylmalonic acid	$C_{16}H_{21}ClO_5$	67	139–140	164.4	164.4

[a] Unstable to heat. [b] Uncorrected. [c] Prepared as an intermediate, Synerholm, M. E., and Zimmerman, P. W., *Contribs. Boyce Thompson Inst.*, **14**, 369(1947).

TABLE III.—ARYLOXYPROPYLCHLOROMALONIC ACID[a]

	Formula	Yield, %	M. P., °C.[c]	Neutral Equivalent Found	Neutral Equivalent Calcd.
γ-(β-Naphthoxy)propylchloromalonic acid[b]	$C_{16}H_{15}ClO_5$	Quant.	138–140, dec.	160.4	161.4
γ-(2,4-Dichlorophenoxy)propylchloromalonic acid	$C_{12}H_{11}Cl_3O_5$	Quant.	130–133	175.6	170.8
γ-(2,4,5-Trichlorophenoxy)propylchloromalonic acid	$C_{12}H_{10}Cl_4O_5$	Quant.	95–98	180.5	188.0
γ-(4-Chloro-2-isopropyl-5-methylphenoxy)propylchloromalonic acid	$C_{16}H_{20}Cl_4O_5$	98	135–140, dec.	178.6	181.9

[a] Unstable to heat. [b] Calcd., C = 59.54%, H = 4.69%; Found, C = 60.39%, H = 4.76%. [c] Uncorrected.

Dilution of the carbon tetrachloride filtrate with low-boiling petroleum ether precipitated a tan solid, m. p. 85° (uncorr.). This compound has not been identified.

Anal. Found: C, 66.74; H, 5.89.

α-Chloro-δ-(4-chloro-2-isopropyl-5-methylphenoxy)valeric Acid.—By the same procedure as in α-chloro-δ-(β-naphthoxy)valeric acid, a dark, gummy oil was obtained from the corresponding chloromalonic acid. The material was not crystallizable from carbon tetrachloride or other organic solvents. Evaporation of the solvents left a lighter-colored oil which was distilled *in vacuo*. The fraction boiling at 217–218° at 3 mm. was retained for a yield of 34%. Neutral eq. calcd. for $C_{15}H_{20}Cl_2O_4$: 319.2. Found: 320.2.

SUMMARY

1. Decarboxylation of the appropriate substituted chloromalonic acids to obtain α-chloro-δ-(β-naphthoxy)valeric acid and α-chloro-δ-(4-chloro-2-isopropyl-5-methylphenoxy)valeric acid has been accomplished.

2. A number of new aryloxypropylmalonic and aryloxypropylchloromalonic acids are reported.

3. Both valeric acids exhibited slight activity in preliminary antifungal screening but were not of sufficient interest for further investigation.

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The Quantitative Determination of Benzylpenicillin by Ultraviolet Absorption*

By EDWARD A. GARLOCK, JR., and DONALD C. GROVE

An ultraviolet spectrophotometric method for the determination of benzylpenicillin in chloroform solution is presented. Separation of active benzylpenicillin from interfering compounds is obtained using the extraction procedure of Boxer and Everett. The active penicillin is extracted at *p*H 2.0 into chloroform and thus separated from basic and neutral material as well as from any chloroform insoluble acidic substances such as penicilloic acid. A correction for any acidic interfering substances extracted with the chloroform is obtained by a separate chloroform extraction of the acidified alkali-inactivated penicillin. The values obtained by assaying commercial samples by this method are compared with those obtained by the official N-ethyl piperidine method.

VARIOUS ultraviolet spectrophotometric techniques for the determination of benzylpenicillin (penicillin G) in the presence of the other naturally occurring penicillins have been reported in the literature. These procedures are based on the measurement of the intensity of the absorption bands due to the phenyl group present in benzylpenicillin. They differ from one another mainly in the techniques used to distinguish between the absorption due to benzylpenicillin and that due to extraneous interfering substances. This interfering absorption can be of two types: (*a*) enhancement of the phenyl "peak" due to phenyl compounds other than benzylpenicillin; and (*b*) background absorption due to compounds exhibiting general absorption.

Levy, Shaw, Parkinson, and Fergus (1) have reported a method utilizing the base-line optical density technique as a means of eliminating interference from background absorption thus limiting interfering substances to phenyl compounds. Grenfell, Means, and Brown (2) have published an ultraviolet absorption method which they report to be extremely accurate on high purity material. Their criteria of purity is that the optical density at 280 mμ must be less than 0.10 for an aqueous solution of the penicillin sample. This 280 mμ optical density which represents small amounts of penicillin decomposition products is deducted from the optical density of the 263 mμ benzyl peak. Colon, Herpich, Neuss, and Frediani (3) have reported a spectrophotometric method employing corrections for two specific common degradation products of penicillin, namely, penicillenic acid and the penaldates

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measured at 320 mμ and 280 mμ, respectively. One-thirtieth of the optical density at 320 mμ and one-third of the optical density at 280 mμ are deducted from the optical density at 257 mμ phenyl peak to give a value corrected for these two impurities. These authors have also made comparative studies of the three spectrophotometric methods described above and report that the method of Grenfell, *et al.*, gives the best agreement with the official F. D. A. precipitation method.

Boxer and Everett (4) have described a colorimetric method for the determination of benzylpenicillin. In this method the penicillin is extracted at *p*H 2.0 into chloroform along with any chloroform-soluble impurities and the total phenyl compounds are determined in the chloroform extract by means of the Kapellar-Adler reaction. A separate portion of the penicillin is converted by treatment with alkali to the penicilloate and this solution likewise extracted at *p*H 2.0 with chloroform. As penicilloic acid is insoluble in chloroform, this extract is used as a blank to correct for any phenyl compounds present other than benzylpenicillin.

It seemed worth while to investigate the possibility of determining the benzylpenicillin in the chloroform extract by ultraviolet absorption instead of the more cumbersome Kapellar-Adler reaction used by Boxer and Everett. Figure 1 shows the ultraviolet absorption of benzylpenicillin as the free acid in chloroform solution of two different concentrations. Inspection of the figure shows a maximum for the phenyl band at 264.5 mμ. Using this wave length a linear relationship was found to exist between optical density and concentrafion of benzylpenicillin (Fig. 2).

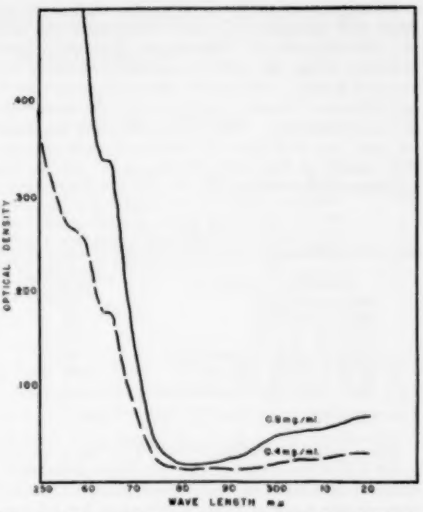

Fig. 1.—Absorption spectrum of benzylpenicillin in chloroform.

PROCEDURE

Weigh 40–50 mg. of the sample to be analyzed into a 25.0-ml. volumetric flask and dilute to the mark with distilled water. Transfer a 5.0-ml. aliquot to each of two 25.0-ml. glass-stoppered test tubes. Inactivate one of the 5.0-ml. aliquots (to be used as the blank) by adding 1.0 ml. of 2 *N* NaOH and allow at least fifteen minutes for inactivation at room temperature. Place the test tubes containing the sample and the inactivated blank in an ice bath and pipette 10.0 ml. of chloroform (previously washed with water) into each tube. After cooling, adjust the *p*H of the solutions to approximately 2.0 by adding 2.0 ml. of a 1:1 mixture of 2 *N* NaOH and 3 *M* H_3PO_4 to the sample and 1.0 ml. of 3 *M* H_3PO_4 to the blank. Shake the tubes throughly for two minutes, centrifuge and withdraw the lower chloroform layer with the aid of a 10.0-ml. hypodermic syringe and a 3.0 inch needle. Superficially dry the chloroform layer by filtering through a pledget of cotton using a U-shaped funnel to reduce evaporation during filtration. Set the spectrophotometer at zero optical density at 264.5 mμ using the blank chloroform extract in a 1-cm. quartz cell. Determine the optical density at 264.5 mμ of the chloroform extract of the sample in a matched 1-cm. quartz cell. A standard curve is prepared by carrying various concentrations of standard benzylpenicillin through the described procedure. The instrument used in this laboratory was a Beckman Model DU Spectrophotometer.

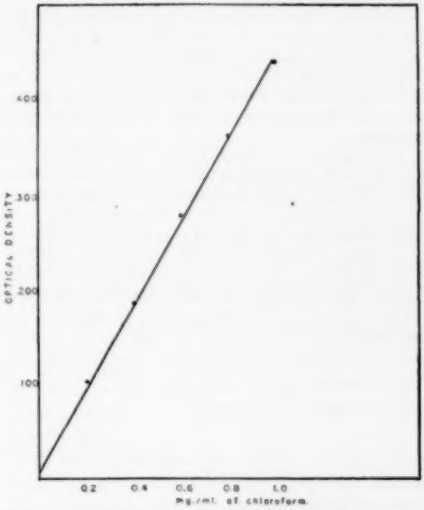

Fig. 2.—Relationship of optical density to concentration of benzylpenicillin in chloroform.

Mixtures were prepared consisting of benzylpenicillin and penicillin K[1] and benzylpenicillin and penicillin dihydro F.[1] The values obtained on these mixtures using the above-described procedure are given in Table I. The results show that penicillins K and dihydro F do not interfere in the determination of benzylpenicillin. Penicillin X was not available for trial, but it should not interfere to any appreciable extent in this method because of its sparing solubility in chloroform.

TABLE I

Composition of Mixture	Penicillin G Found, %
90% G–10% K	91.0
80% G–20% K	81.0
70% G–30% K	69.5
90% G–10% Dihydro F	88.5
80% G–20% Dihydro F	81.0
70% G–30% Dihydro F	72.5

When benzylpenicillin, which had been decomposed with sodium hydroxide, was added to a known quantity of standard benzylpenicillin and carried through the procedure, the recovery of benzylpenicillin was low. This same phenomenon was noted when acid degradation products were added. Our only explanation for this is that the presence of decomposition products caused a rapid degradation in solution of the active benzylpenicillin while the procedure was being carried out.

A series of commercial samples of penicillin G were assayed by this procedure and by the N-ethyl piperidine gravimetric (N. E. P.) method (5) in current use. The results are shown in Table II.

The results obtained by this ultraviolet method are in general somewhat higher than those obtained by the N-ethyl piperidine method. A similar difference has been previously observed when an infrared method (6) was compared with the N-ethyl piperidine method.

It is believed that the N-ethyl piperidine method tends to give low results, and values obtained by the ultraviolet method for benzylpenicillin are closer to the true values. The ultraviolet method is simple and rapid to carry out and is not subject to the vagaries encountered in the N-ethyl piperidine method.

The ultraviolet method as described is not applicable to the determination of the benzylpenicillin content of procaine penicillin. This is due to the fact that a small amount of procaine, which has a high absorption at 264.5 mμ, is extracted into the chloroform. It is believed that by proper modifications of the procedure this difficulty can be eliminated. Such modifications are being investigated.

[1] Supplied through the courtesy of Chas. Pfizer and Co., Brooklyn, N. Y.

TABLE II

Manufacturer	Type Salt	U. V., %	Penicillin G, N. E. P., %	Difference, U. V. − N. E. P., %
A	Na	95.5	89.8	+5.7
	Na	89.0	92.2	−3.2
B	Na	98.5	96.0	+2.5
	Na	98.5	95.8	+2.7
C	Na	99.5	94.8	+4.7
	Na	100.0	100.1	−0.1
D	Na	97.0	90.2	+6.8
	Na	101.0	93.8	+7.2
	K	99.0	91.2	+7.8
	K	100.5	92.5	+8.0
E	Na	97.0	99.5	−2.5
	Na	96.0	92.6	+3.4
F	K	91.0	84.9	+6.1
	K	96.0	86.7	+9.3
G	Na	100.5	95.1	+5.4
	Na	99.0	89.7	+9.3
H	K	99.0	95.5	+3.5
	K	103.5	100.1	+3.4
I	Buffered K	89.0	91.1	−2.1
	Buffered K	95.5	94.0	+1.5
J	Buffered K	94.5	93.8	+0.7
	Buffered K	93.5	97.6	−4.1
		Average difference		+3.3

We have not applied this ultraviolet method to the determination of the benzylpenicillin content of fermentation broths. It seems likely, though, that the method or a modification of it might be satisfactory for this purpose.

SUMMARY

Benzylpenicillin can be satisfactorily determined by ultraviolet absorption using a solvent extraction procedure similar to that of Boxer and Everett (4). In this procedure a blank determination is used to correct for interfering substances.

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The Preparation and Study of Some Thiazole Derivatives of Antimony*,†

By DONALD B. MEYERS‡ and JAMES W. JONES§

Preparative methods for some organic antimony compounds are discussed. The preparation of thiazole-2-stibinic tetrachloride and its use as an intermediate in production of others is outlined. The oral and intraperitoneal acute toxicities of the sodium salt of thiazole-2-stibinic acid in the white rat are reported. The results of an investigation of the effect of that compound on the adult *Trichinella spiralis* in the gastrointestinal tract of the white rat indicate that it is ineffective in a sufficiently low dosage to be nontoxic.

THE history of the use of antimony as a therapeutic agent is an ancient one. Many references to the use of stibium salts in medicine have been found through the centuries, some dating back before the time of Christ. However, the true place of antimony as a therapeutic agent in many protozoan diseases was not discovered until 1907, when Plimmer and Thomsen (1) published their findings on the use of antimony as a trypanocidal agent.

Soon after the publications on the use of antimonyl potassium tartrate in trypanosomiasis, came the discovery by Di Cristonia and Caronia (2) that the same drug was highly effective in the treatment of kala-azar. Today it is recognized that intravenous injections of tartar emetic are highly effective against a number of tropical diseases due to animal parasites, especially against kala-azar, bilharziasis, Delhi boil, filariasis, and granuloma inguinale, while good results have been obtained in leishmaniasis, sleeping sickness, and frambesia (3).

Since the recognition of the therapeutic value of tartar emetic, a number of pentavalent and trivalent antimonials have been prepared in an attempt to find a highly effective compound with a low toxicity. A review of the literature reveals that a great number of antimonyl stibinic, stibinous, and stibino compounds have been prepared and studied. It was soon discovered that a compound which was decidedly effective in combating one type of protozoa was quite inferior to some other antimonial when applied to a second type of organism. Also the organic part of the molecule was shown to greatly influence the toxicity of the compound to the host.

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The first true organic (C—Sb) compounds were prepared in the latter half of the 19th century. These were aliphatic derivatives and showed no promise as therapeutic agents (4–6).

In 1882, Michaels and Reese (7) published a paper on the preparation of aromatic stibines, but it was not until 1912 (8) that it was discovered that aromatic stibinic compounds could be prepared in good yield through the diazo reaction. This reaction was further developed by Schmidt (9) who, in 1920, released an excellent series of papers covering the preparation of numerous aryl stibinic acids.

The purpose of this investigation was to prepare a series of compounds in which antimony was attached directly to the thiazole ring and to determine if they showed any activity against the adult *Trichinella spiralis* in the gastrointestinal tract. In earlier work we (10) prepared antimonyl 2-aminothiazole tartrate and studied its toxicity. It was thought that antimony thiazole compounds might have some therapeutic advantage over the phenyl derivatives as antiparasiticidal agents. Though the true evaluation of thiazole in therapeutics has not been fully determined, it is known that the thiazole ring occurs in at least two important naturally occurring medicinals, namely, thiamine and penicillin.

Gould (11) points out that a drug to be effective in combating the *Trichinella* parasite must act: (*a*) on the adult worm in the intestine; (*b*) on the larvae during migration; or (*c*) on the larvae during muscle invasion and encystment. Despite the great amount of work that has been devoted to this problem no therapeutic agent that is effective in any of these stages has been developed.

If action on the adult worm in the gastrointestinal tract is desired, the properties considered necessary for any chemotherapeutic agent used in combating helminths are:

(1) A relatively low solubility in the medium of the gastrointestinal tract so that systemic effects are reduced to a minimum.

(2) A low index of irritation to the stomach and intestinal walls without nausea.

(3) A highly effective antiparasiticidal action.

(4) A high therapeutic index; that is, a wide range between the therapeutic dose and the dose toxic to the host.

(5) Stability of the drug to heat, dampness, etc.

On the other hand if the medicinal is to be given for systemic treatment, it must be soluble in aqueous media and should be nonirritating when injected. Thiazole derivatives of antimony offer both water-soluble and water-insoluble compounds.

A review of the procedures employed for obtaining organic antimony derivatives showed that the diazotization method was the most suitable for obtaining monoaromatic preparations. Although thiazole is a heterocyclic compound, it has sufficient aromatic properties so that its 2-amino derivative will undergo diazotization under suitable conditions. This aromatic behavior can be attributed to the presence of both a sulfur atom and a nitrogen atom in the ring. Morgan and Morrow (12) showed that 2-aminothiazole could be diazotized in the presence of the oxy-acids but attempts to diazotize the compound in concentrated hydrochloric acid usually produced 2-chlorothiazole, the unreactive diazohydroxide or thiazole itself. They also stated that the hydrochloride salt of 2-aminothiazole had little tendency to undergo diazotization. However, it was found in this investigation that the sulfate salt could be diazotized with ethyl nitrite if the proper temperature conditions were maintained.

Standard procedures generally employed for preparing aromatic stibinic compounds by the diazotization method were of no value in preparing the stibinic derivatives of thiazole. Schmidt's (9) method, by which phenyl stibinic acid was obtained through the diazotization of aniline in concentrated hydrochloric acid, was much too drastic. The 2-chloro derivative of thiazole was the chief product obtained when this method was applied to 2-aminothiazole. A small yield of thiazole-2-stibinic acid was obtained when 2-aminothiazole was treated with nitrosyl sulfuric acid in the cold, followed by the addition of antimonous trioxide in dilute alkali. The product was very impure and difficult to free from the colored matter which it contained.

The method of Binz and Schickh (13), which gave good yields of the hydrochloride of pyridine-3-stibinic tetrachloride from 3-aminopyridine, was not applicable to use with 2-aminothiazole, which is an isoster of 2-aminopyridine. No better yields were obtained when sulfuric or nitric acid was substituted for the hydrochloric acid.

EXPERIMENTAL

The following procedure, using ethyl nitrite in the presence of antimony trichloride, was the only one found to give satisfactory results in the preparation of stibinic derivatives of thiazole.

Preparation of Thiazole-2-stibinic Tetrachloride.—Twenty grams of 2-aminothiazole was dissolved in 200 cc. of ethanol in a liter beaker and 20 Gm. of concentrated sulfuric acid was slowly added. The precipitate which formed after addition of the first portion of the acid redissolved after the total amount of the acid had been added. Fifty grams[1] of antimonous trichloride in 200 cc. of ethanol was poured into the first solution and the mixture was cooled to 0° in an ice bath. Twenty cubic centimeters of ethyl nitrite was then added in several portions over a period of twenty minutes, during which time the mixture was constantly stirred with a mechanical stirrer. The temperature of the mixture was then allowed to rise slowly to 25° and stirring was continued at this temperature for forty-eight hours, during which time there was a slow evolution of nitrogen. At the end of this period the mixture was heated to 60° for one hour, cooled, and filtered. The filtrate was then evaporated to a volume of 200 cc. and placed in the cold. In a few hours the precipitation of almost pure crystals of thiazole-2-stibinic tetrachloride occurred. The crystals were then dissolved in 200 cc. of warm ethanol. Five grams of charcoal was added, the mixture boiled for two minutes, and filtered. The filtrate was allowed to stand in the cold until the crystals again precipitated. The crystals were then filtered from the alcoholic solution, dried, and recrystallized from boiling ethanol. The total yield of thiazole-2-stibinic tetrachloride was 55%, m. p. 133°.

Anal.—Calcd.: Sb, 35.01%; Cl, 40.78%; S, 9.21%. Found: Sb, 34.72%; Cl, 40.63%; S, 9.11%.

Thiazole-2-stibinic tetrachloride is soluble in methyl alcohol, ethyl alcohol, acetone, and dilute hydrochloric acid. It decomposes in water to give a white, insoluble compound which is probably a mixture of the oxychlorides which result from partial hydrolysis of the compound. Dilute alkali converted the chloride to a soluble stibinic acid salt.

This compound was prepared in similar manner, starting with the isolated 2-aminothiazole sulfate salt but isolation of the sulfate was of no advantage in increasing the yield or purity of the original precipitate of thiazole-2-stibinic tetrachloride.

The reactions involved in the preparation of thiazole-2-stibinic tetrachloride may be represented in two steps as follows:

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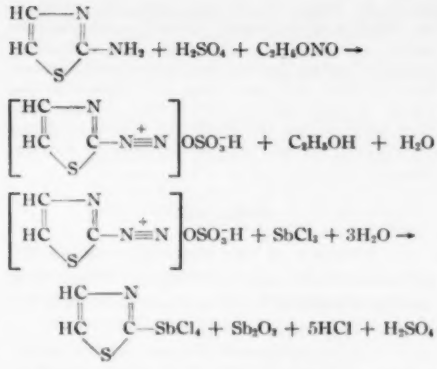
[1] A greater amount did not increase the yield of the thiazole-2-stibinic tetrachloride.



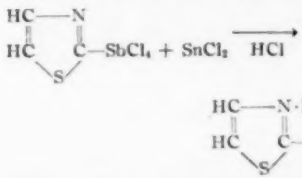
Preparation of Thiazole-2-stibinous Chloride Hydrochloride.—Six grams of thiazole-2-stibinic tetrachloride was mixed intimately with 20 Gm. of stannous chloride. To this mixture, 60 cc. of ethanol saturated with dry hydrochloric acid was added. As the reaction proceeded, the mixture became warm and the solid components went into solution. After fifteen minutes the solution was placed in the cold, and colorless crystals of thiazole-2-stibinous chloride hydrochloride precipitated. The crystals were separated from the solution onto a filter paper, dried, and recrystallized from alcohol saturated with hydrochloric acid. They were then recollected on a filter and dried in vacuum. The total yield was 4.2 Gm. or 80%, m. p. 138°.

Anal.—Calcd.: Sb, 38.86%; Cl, 33.93%. Found: Sb, 38.51%; Cl, 33.72%.

The compound was also prepared as follows: 6 Gm. of thiazole-2-stibinic tetrachloride was dissolved in 50 cc. of absolute methanol saturated with dry hydrochloric acid by warming the mixture slightly. One-half gram of potassium iodide was added and the solution saturated with sulfur dioxide. The mixture was allowed to stand in the cold for twelve hours. The crystals of thiazole-2-stibinous chloride hydrochloride which precipitated from the cooled solution were collected on a filter and dried in vacuum.

Thiazole-2-stibinous chloride hydrochloride is soluble in methyl or ethyl alcohol, acetone, and dilute hydrochloric acid. It is converted to the corresponding stibinous oxide by treatment with dilute alkali.

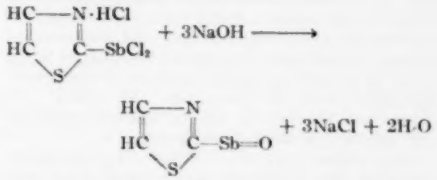
The reactions involved in the reduction of the stibinic tetrachloride were as follows:



Preparation of Thiazole-2-stibinous Oxide.—Five grams of thiazole-2-stibinous chloride hydrochloride was placed in 20 cc. of cold water, and 1.6 Gm. of sodium hydroxide in 30 cc. of water was added. The mixture was warmed gently and a white powder-like precipitate of thiazole-2-stibinous oxide resulted. The powder was filtered from the solution, washed with several portions of distilled water, and recrystallized from glacial acetic acid by the slow addition of water. The yield was 4 Gm. or 95%, m. p. 250°.

Anal.—Calcd.: Sb, 54.87%. Found: Sb, 54.78%.

Thiazole-2-stibinic oxide is almost insoluble in water, alcohol, acetone, or ether. It is soluble in glacial acetic acid.



Toxicity of the Sodium Salt of Thiazole-2-stibinic Acid to White Rats.—The acute toxicity, both oral and intraperitoneal, of the sodium salt of thiazole-2-stibinic acid was determined on white rats. Freshly prepared thiazole-2-stibinic tetrachloride was dissolved in distilled water containing just enough sodium hydroxide to convert it to sodium thiazole-2-stibinate. Enough distilled water was added so that each cubic centimeter represented 50 mg. of the salt. The resulting solution had a *p*H of 8.2.

The intraperitoneal injections were made with a hypodermic syringe in the regular manner in graded dosages of 100–1000 mg. of drug per Kg. of rat. As can be seen from Table I, the approximate LD_{50} was 310 mg./Kg.

TABLE I.—ACUTE TOXICITY OF INTRAPERITONEAL INJECTIONS OF SODIUM THIAZOLE-2-STIBINATE IN ALBINO RATS

Dose Drug, Mg./Kg.	Sb, Mg./Kg.	No. Rats	No. Alive	No. Dead	% Survival
100	42.5	5	5	0	100.00
200	85	12	10	2	83.33
400	170	12	4	8	33.33
800	340	5	1	4	20.00

The oral dosages were administered by means of a small glass stomach pipette. Each rat was anesthetized with ether, held just below the jaws, and the pipette was inserted down the esophagus into the stomach where the drug was deposited. The oral toxicity was not as great as the intraperitoneal. The computed oral LD_{50} of sodium thiazole-2-stibinate was found to be approximately 750 mg./Kg. (see Table II).

TABLE II.—ACUTE TOXICITY OF ORAL DOSES OF SODIUM THIAZOLE-2-STIBINATE IN WHITE RATS

Dose Drug, Mg./Kg.	Sb, Mg./Kg.	No. Rats	No. Alive	No. Dead	% Survival
200	85	3	3	0	100.00
400	170	6	6	0	100.00
600	275	12	11	1	91.66
800	340	12	5	7	41.66
1000	425	3	0	3	0.00

Only rats which died within twenty-four hours after administration of the drug were considered to have died from acute poisoning. The intestinal wall of rats killed by the oral dose showed signs of definite irritation but no acute lesions were observed.

Action of Sodium Thiazole-2-stibinate on the Adult *Trichinella spiralis* in the Gastrointestinal Tract of the Albino Rat.—No attempt was made to undertake an extensive pharmacological study of any of the compounds whose preparation is discussed in the forepart of this paper. However, the sodium salt of thiazole-2-stibinic acid was screened to see if it had any action against the adult or larval stage of *Trichinella spiralis* infection in the gastrointestinal tract of white rats. As Gould (11) points out, there is, as yet, no effective agent for the specific treatment of trichinosis.

The method used for infecting the rats and in counting the *Trichinella* was adopted from that employed by Gursch and Nolf (14). Doses of 2000 larvae were prepared by dilution count and concentrated by centrifugation. By means of a small pipette, which was inserted directly through the mouth into the stomach, the larvae were administered to anesthetized rats in such sequence that there was ample time to complete individual experiments. At the same time the recorded amount of drug was administered by means of an identical pipette. After seventy-two hours each rat was killed and the entire intestine was removed, taking precautions to keep it at body temperature during the process. The intestine was then cut into two nearly equal parts and each part flushed thoroughly with warm water from a bulb pipette, the washing being collected in a liter flask. The two portions were then cut open and the mucosa was stripped from the muscularis and added to previous washings. Ten cubic centimeters of Tergitol 0.7 (a synthetic surface tension reducer) was added and the number of adult worms present determined by direct count.

As can be seen by Table III, doses of sodium thiazole-2-stibinate which could be considered safe for administration had little or no effect on the adult *trichinae*. Doses of the drug equivalent to about one-third of the computed LD_{50} dose were partially effective in killing some of the worms but such a dose killed 20% of the rats to which it was administered. One rat from each dose range was allowed to live for six to eight weeks after infection. They were then sacrificed and the diaphragm muscle examined for encysted larvae. All showed positive encystation.

TABLE III.—EFFECT OF ORAL ADMINISTRATION OF SODIUM THIAZOLE-2-STIBINATE ON THE ADULT *Trichinella spiralis*

No. Larvae Administered	Drug Dose, Mg./Kg.	Sb, Mg./Kg.	No. Rats	No. of Rats in Which the Adult Worm Count Was >400	<400	Absent
2000	0	0	10	8	2	0
2000	50	29	10	9	1	0
2000	100	42	10	7	3	0
2000	250	105	10	4	2	2[a]

[a] Two rats killed by drug.

SUMMARY

1. A review of the literature covering the preparation of organic antimony compounds and the reported effect of some of them on infections of *Trichinella spiralis* was made.

2. The preparation of thiazole-2-stibinic tetrachloride and some of the other thiazole antimony compounds which can be prepared from it has been outlined.

3. The oral LD_{50} of one of these compounds, the sodium salt of thiazole-2-stibinic acid, was found to be 310 mg./Kg. in the white rat while the intraperitoneal LD_{50} was shown to be 750 mg./Kg.

4. It was shown that sodium thiazole-2-stibinate had little effect on the adult *Trichinella spiralis* in the gastrointestinal tract of the white rat if the dosage was low enough to be safe for administration.

5. It is hoped that the parasiticidal investigation can be extended to determine the effect of the other compounds of antimony described herein, especially that of thiazole-2-stibinous chloride.

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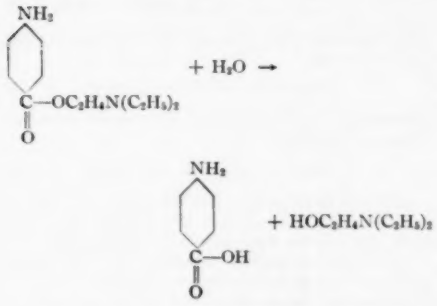
The Kinetics of the Hydrolysis of Procaine*,†

By TAKERU HIGUCHI, ANNA HAVINGA,‡ and L. W. BUSSE

Procaine solutions are subject to hydrolytic deterioration. In the present paper results of a chemical kinetical investigation of this reaction together with its theoretical analysis are given. It is shown that the over-all reaction is the sum of two reactions involving the free base and the singly charged ion. The activation energies have been found to be 13.8 and 12 kilocalories per mole, respectively, for the two hydrolytic reactions. From the data obtained general equations relating the half life of procaine solutions to their temperature and *p*H are derived.

INSTABILITY of procaine in solution is due mainly to the hydrolysis of the ester linkage in the compound according to the equation



yielding *p*-aminobenzoic acid and diethyl ethanol amine.[1] This reaction appears to be primarily base-catalyzed, the ester being relatively resistant toward acid-catalyzed hydrolysis.

Although the literature on the rate of hydrolysis of procaine solutions is voluminous, no attempt seems to have been made to study the problem from a chemical kinetics point of view. Investigations were generally of the type which permitted simultaneous variation of the ester concentration, hydrogen ion concentration, and temperature. For this reason it is practically impossible to apply the data available in the literature except to the particular systems studied.

In the present investigation an attempt has been made to apply the methods of classical chemical kinetics to the hydrolytic reaction of procaine. By successively keeping all but one variable essentially constant, it has been possible to resolve the over-all effect into the individual contributing factors. Studies were made at several constant hydroxyl ion concentrations covering roughly a million-fold difference in concentration. Rate measurements were also carried out in temperatures ranging from 10° to 82°. The influence of various buffer types on the velocity of the hydrolysis reaction was also made. From the various data obtained, heats of activation and a general rate equation which appeared to be valid over widely differing conditions were derived.

THEORETICAL CONSIDERATION

As a rule esters are subject to both hydrogen ion and hydroxyl ion catalyzed hydrolysis. Suggestions have been made that many reactions of this type, which are susceptible to proton and hydroxyl ion catalysis, are also subject to general acid and base catalysis. However, hydrolysis of esters seems to be essentially specifically catalyzed only by hydrogen and hydroxyl ions (in their hydrated form); no substantiated case of catalysis by undissociated acid or base being demonstrable (1). There is no reason to believe that procaine would be an exception to this rule.

The problem of procaine hydrolysis is further simplified by the fact that the rate of proton-catalyzed reaction is very slow. Even in presence of moderately concentrated acid (e.g., 0.1 *N* HCl) hydrolysis proceeds relatively slowly even at autoclave temperatures (2). Since nearly all commercial procaine preparations have *p*H values over 5, it is evident that, for all practical purposes, the acid-catalyzed hydrolysis takes no part in the deterioration of the drug.

This then leaves the hydroxyl ion catalyzed reaction as the most probable mechanism of hydrolysis. Several reaction pathways, however, are still available. Since procaine can occur in three different forms in solution, i.e., as the free base, as the singly charged ion, or as the doubly charged ion, the reaction mechanism may be expected to change with the *p*H of the solution. At 25° and above *p*H of 9, the the drug is present mainly as the free base, between *p*H of 9 and 2.3 as the half salt, and below a *p*H of 2.3 as the completely neutralized salt. If we assume that the rate of hydrolysis of the doubly charged procaine ion is not of consequence (this will be borne out experimentally later in this paper) we can then postulate the following principal hydrolytic reactions (see page 406) where K_1 and K_2 are the respective constants for the equilibria given, and k_1^* and k_2^* are the respective reaction constants. For the sake of simplicity it is convenient to set $k_1 = K_1k_1^*$ and $k_2 = K_2k_2^*$.

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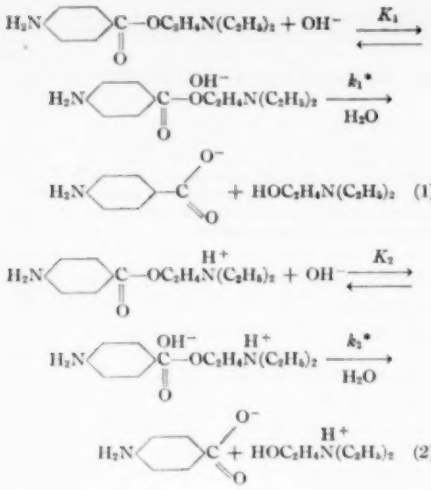
† Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.
‡ American Foundation for Pharmaceutical Education Fellow, 1948–1949.
[1] The ester is also subject to oxidative breakdown but this source of deterioration can be controlled by removal of oxygen and addition of an antioxidant to the solution.



Or if "Pr" represents the concentration of the base form and "PrH^+" the concentration of the ionic form, we have

$$\frac{d(Pr)}{dt} = k_1(OH^-)(Pr) \quad (1)$$

and

$$\frac{d(PrH^+)}{dt} = k_2(OH^-)(PrH^+) \quad (2)$$

Both Pr and PrH^+ can be expressed in terms of the total drug concentration, a, hydroxyl ion concentration, and the dissociation constant of the base, k_b:

$$(Pr) = \frac{a(OH^-)}{k_b + OH^-} \quad (3)$$

and

$$(PrH^+) = \frac{ak_b}{k_b + (OH^-)} \quad (4)$$

If we assume that the over-all rate of hydrolysis is the sum of the individual rates, we get

$$\frac{da}{dt} = \frac{d(Pr)}{dt} + \frac{d(PrH^+)}{dt} = k_1(OH^-)(Pr) + k_2(OH^-)(PrH^+) \quad (5)$$

Substituting Eqs. (3) and (4) into (5), we obtain

$$\frac{da}{dt} = k_1(OH^-)\frac{a(OH^-)}{k_b + (OH^-)} + k_2(OH^-)\frac{ak_b}{k_b + (OH^-)} \quad (5)$$

or

$$\frac{d \ln a}{dt} = \frac{(OH^-)}{k_b + (OH^-)}[k_1(OH^-) + k_2k_b] \quad (6)$$

It is evident on inspection of the right-hand part of the above equation that it is a constant for any given hydroxyl ion concentration. Accordingly if Reactions 1 and 2 are primarily responsible for the hydrolysis of procaine, the over-all rate of hydrolysis would be first order with respect to the drug concentration. Thus, at any constant hydroxyl ion concentration the half life of the drug would be related to the hydroxyl ion concentration according to the relationship

$$\frac{0.693}{t_{1/2}} = \frac{(OH^-)}{k_b + (OH^-)}[k_1(OH^-) + k_2k_b]$$

If we define $k'_1 = \frac{k_1}{0.693}$ and $k'_2 = \frac{k_2}{0.693}$, we have instead

$$\frac{1}{t_{1/2}} = \frac{(OH^-)}{k_b + (OH^-)}[k'_1(OH^-) + k'_2k_b] \quad (7)$$

The above equation can also be expressed logarithmically,

$$-\log t_{1/2} = \log(OH^-) - \log[k_b + (OH^-)] + \log[k'_1(OH^-) + k'_2k_b] \quad (8)$$

At a relatively high hydroxyl ion concentration Eq. (8) reduces to

$$-\log t_{1/2} = \log(OH^-) + \log k'_1 \quad (9)$$

Conversely, at low hydroxyl ion concentration the equation simplifies to

$$-\log t_{1/2} = \log(OH^-) + \log k'_2 \quad (10)$$

If our original assumptions hold true, it is evident from Eqs. (9) and (10) that at both ends of the pH scale the rate of hydrolysis (or, equivalently, the reciprocal of the half life) would be directly proportional to the hydroxyl ion concentration. In the intermediate region there would be, however, a region of nonproportionality.

EXPERIMENTAL

The experimental method employed in the present study consisted essentially of making a series of ultraviolet adsorption measurements on a procaine solution undergoing hydrolysis in a constant temperature bath.

Ultraviolet Analysis.—Although the ultraviolet absorption spectra of procaine and its chromophore, containing hydrolytic product, p-aminobenzoic acid are practically identical, the salt form of the acid has sufficiently different electronic configuration so that its absorption spectrum is different enough to permit quantitative analysis of mixtures of the salt and the ester. The ultraviolet absorption curves are shown in Fig. 1.

The following equation based on application of the Beer's law relationship to binary mixtures was used to calculate the degree of hydrolysis:

$$\%\text{ residual ester} = 100 \times \frac{(k - k_{PABA})}{(k_{proc.} - k_{PABA})}$$

where

k = the observed optical density of the partly hydrolyzed sample diluted to 0.00100% with respect to original procaine hydrochloride

k_{PABA} = the optical density of p-amino benzoate ion equivalent to 0.00100% concentration of procaine hydrochloride

$k_{proc.}$ = the optical density of 0.00100% procaine hydrochloride, all readings being taken at a fixed wave length, and pH of 9.5

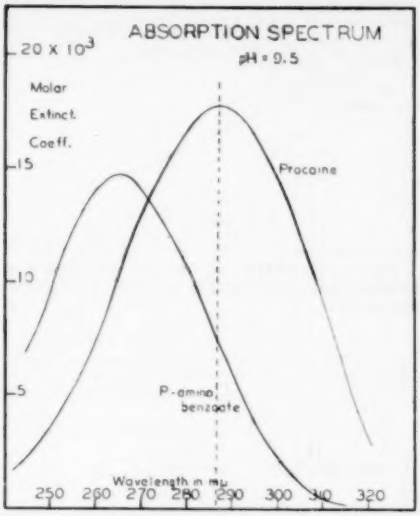


Fig. 1.—Ultraviolet absorption curves of procaine and *p*-aminobenzoic acid in water at *p*H of 9.5.

The absorption measurements were taken on a Beckman Model DU Quartz Spectrophotometer at wave lengths of 287 mμ and 271.5 mμ. The values obtained at the first wave length which corresponded to the first absorption peak of procaine were used to calculate the degree of hydrolysis. The second wave length readings were theoretically time independent since the molar extinction coefficient of the ester and the acid produced are identical in alkaline solution, thus constituting an internal check on any side reaction which may take place. This proved rather useful in restricting the data-taking time to those parts of the reaction relatively free from the oxidative reaction.

Buffer System.—To maintain the hydroxyl ion concentration essentially constant during the course of the acid-producing hydrolytic reaction, buffers or large excesses of strong bases were employed. The largest change in the hydroxyl ion concentration observed during all the runs made was 0.2 expressed in *p*OH unit.

Although both acid and base buffers were employed, the latter type was preferred because of negligible change in the maintained hydroxyl ion concentration with temperature. With the acid type, e.g., phosphate buffers, the hydrogen ion concentration is essentially independent of temperature, but because of the relatively large temperature coefficient of the ionic product, $(OH^-)(H)$, of water, the maintained hydroxyl ion concentration is a relatively sensitive function of temperature. These were nevertheless also used because of difficulty in obtaining proper *p*H coverage with the base-type buffers alone.

Borate, phosphate, citrate, and ammonium buffers were used in this study as listed in Table I. In all cases, *p*H of the buffers was determined at the temperature of the run.

Experimental Procedure.—The following procedure with only minor modifications was employed in the present investigation:

A 100-ml. volumetric flask containing slightly less than 90 ml. of buffer solution was placed in a thermostatically controlled bath. When the flask content had reached the temperature of the bath, exactly 10 ml. of a 1% procaine hydrochloride solution was added. The flask was then filled to the mark with buffer solution and shaken. The progress of the reaction was followed by sampling at certain intervals depending on the rate of hydrolysis.

The withdrawn samples were diluted one hundredfold with chilled, boiled, distilled water and simultaneously adjusted to *p*H of 9.5 with 0.1 *N* sodium hydroxide. The optical densities of the resulting solutions were determined at wave lengths of 287 and 271.5 mμ on a Model DU Beckman Spectrophotometer.

In some runs, especially those made above *p*H of 9, lower procaine concentrations were used due to the tendency of the drug to precipitate out under such conditions. In such cases the extent of dilution was modified to yield the same final concentration for spectrophotometric analysis.

RESULTS AND DISCUSSION

Influence of the Ester Concentration.—As may be expected the observed rate of cleavage of procaine at any given temperature and hydroxyl ion concentration is first order with respect to the ester concentration. This is illustrated in Figs. 2, 3, and 4 representing neutral, slightly alkaline, and moderately alkaline hydrolysis conditions, respectively. This is in accord with Eq. (6).

TABLE I.—LIST OF BUFFERS EMPLOYED AND COMPOSITION OF EACH

*p*H	Composition	Dilution
5.00	48.5 ml. 0.1 *M* citric acid + 51.5 ml. 0.2 *M* Na_2HPO_4	As is
6.10	36.85 ml. 0.1 *M* citric acid + 63.15 ml. 0.2 *M* Na_2HPO_4	As is
6.55	30.00 ml. 0.1 *M* citric acid + 70.00 ml. 0.2 *M* Na_2HPO_4	As is
7.00	38.90 ml. 1/15 *M* KH_2PO_4 + 61.1 ml. 1/15 *M* Na_2HPO_4	As is
8.00	5.50 ml. 1/15 *M* KH_2PO_4 + 94.5 ml. 1/15 *M* Na_2HPO_4	As is
8.00	100 ml. 2 *M* NH_4OH + 95.20 ml. 2 *M* HCl	To 1 liter
8.25	100 ml. 2 *M* NH_4OH + 91.81 ml. 2 *M* HCl	To 1 liter
8.45	100 ml. 2 *M* NH_4OH + 86.30 ml. 2 *M* HCl	To 1 liter
9.00	100 ml. 2 *M* NH_4OH + 66.67 ml. 2 *M* HCl	To 1 liter
9.50	100 ml. 2 *M* NH_4OH + 36.15 ml. 2 *M* HCl	To 1 liter
9.90	100 ml. 2 *M* NH_4OH + 10.66 ml. 2 *M* HCl	To 1 liter
10.45	100 ml. 2 *M* NH_4OH + 5.93 ml. 2 *M* HCl	To 1 liter
9.00	90 ml. 0.05 *M* borax + 10 ml. 0.2 *M* boric acid	As is
8.45	50 ml. 0.05 *M* borax + 50 ml. 0.2 *M* boric acid	As is

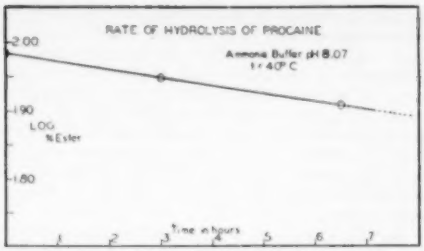


Fig. 2.—Hydrolysis of procaine in solution at *p*H of 8.07 and at 40°.

Influence of Hydroxyl Ion Concentration.—The dependency of the rate of hydrolysis (expressed in terms of the half life in minutes of the drug) on the hydroxyl ion activity is shown in Fig. 5. To permit comparison under comparable conditions all the experimental points shown in the figure were either determined (circles) at 40° or, if the measurements were taken at higher or lower temperatures, were corrected (crosses) to 40°. It was not possible to cover the entire range of hydroxyl ion concentration shown ($p\mathrm{OH} = 1 - 7$) directly at this temperature since the half life of the drug varied roughly ten thousand-fold over this range of concentration. The temperature corrections are discussed later in this article.

The smooth curve which apparently fits the experimental values was not drawn through the points but rather represents Eq. (8) where

$$k'_1 = 0.95 \text{ min.}^{-1}$$
$$k'_2 = 270 \text{ min.}^{-1}$$
$$k_b = 1.6 \times 10^{-8}$$

The values for k'_1 and k'_2 were obtained by substituting in Eqs. (9) and (10), respectively, the extreme experimental values shown in the curve. The constant, k_b, was calculated from the *p*H of a half-neutralized solution of procaine at 40° containing 1% NaCl.

The rather nice fit between the predicted relationship and the experimental points indicates that the hydrolytic reactions 1 and 2 or their equivalents are mainly responsible for the hydrolysis of procaine in solution. The experimental data obtained support our original assumption as to the role played by the doubly charged procaine ions. It is evident that if reaction such as (see next column):

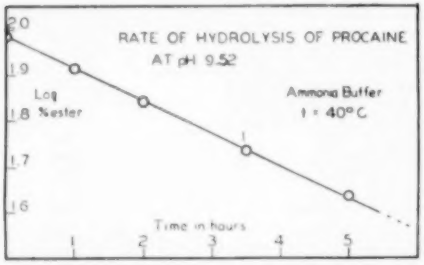


Fig. 3.—Hydrolysis of procaine in solution at *p*H of 9.52 and at 40°.

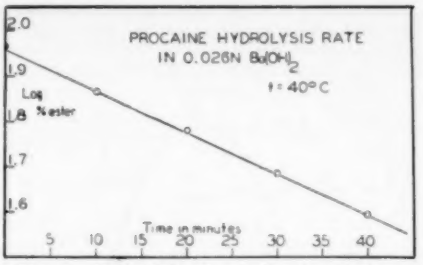
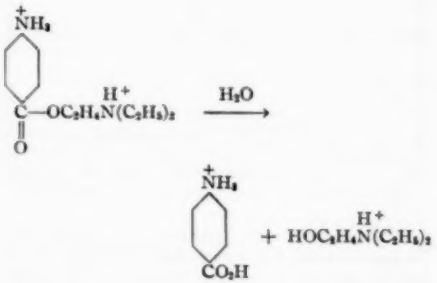


Fig. 4.—Hydrolysis of procaine in solution at hydroxyl ion concentration of 0.026 *N* [$Ba(OH)_2$] and at 40°.



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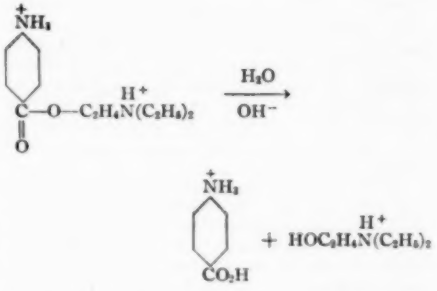

were significant in the hydrolysis of procaine, the over-all reaction rate at the lower *p*H ranges studied would not have shown the direct proportionality to hydroxyl ion concentration as actually found.

Since the validity of Eq. (8) seems to be independent of buffer type and concentration, it would appear that the first order rate constant is dependent only on the hydroxyl ion activity and the temperature of the solution. Since potentiometric measurements determine the hydroxyl ion activity rather than concentration, the former, in view of the excellent correlation obtained, is probably the important one. This can be rationalized by postulating an equilibrium reaction involving hydroxyl ions as preceding the rate-determining step. This is in accord with both Reactions 1 and 2.

In many instances even the best physical chemical equations are applicable over only a limited range of variables. It is rather remarkable that a kinetics

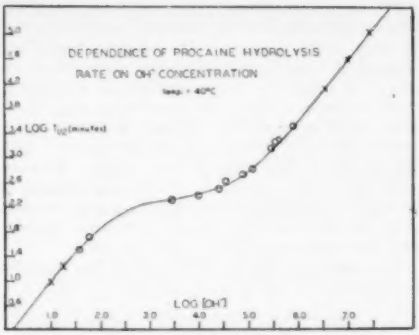

Fig. 5.—A curve relating the half life of procaine with the hydroxyl ion content of the reaction mixture. The solid line represents the relationship

$$-\log t_{1/2} = \log(OH^-) - \log\left[\frac{1.6 \times 10^{-8}}{(OH^-)} + 1\right] + \log\left[0.95 + \frac{4.3 \times 10^{-3}}{(OH^-)}\right].$$

Circles represent experimental points determined at 40°. Crosses are experimental points determined at temperatures other than 40° and corrected to 40°.

equation such as Eq. (8) should be valid over a million-fold variation in catalyst concentration with a maximum error of less than 20%.

Temperature Dependency of Hydrolysis.—Since the hydrolysis reaction may proceed by either of the two pathways, Reaction 1 and 2, it was necessary in determining the temperature dependency of the hydrolysis rate to ensure that the measurements were essentially of one or the other, not of a mixture of the two. For this reason a temperature-dependency study was first conducted on a very alkaline solution (0.026 *N*) such that Reaction 1 contributed nearly entirely to the over-all rate. The conditions were then changed to a lower *p*H so that Reaction 2 was essentially responsible for the over-all hydrolysis.

Determination of Temperature Dependency of Reaction 1.—Series of half-life measurements were made on a solution which was 0.026 *N* with respect to hydroxyl ion. Since 0.100% procaine solutions or less were used, less than 10% of the base was consumed up to the 50% hydrolysis point. Within the precision of the present study it was assumed, therefore, that the hydroxyl ion concentration was essentially constant. In obtaining the half lives, it was necessary to resort to a refrigerated constant temperature bath since the hydrolysis rates were extremely high, much above room temperature.

The results obtained are shown in Fig. 6 where the logarithm of the observed half lives are plotted against the reciprocal of the respective absolute temperatures. The heat of activation calculated from the slope of the line is 13.8 kilocalories with a probable error of about a kilocalorie. And since the first order rate constant = $0.693/t_{1/2}$ we can solve for *s* or the frequency factor in the Arrehnius equation

$$k = se^{-\left(\frac{\Delta H_a}{RT}\right)}$$

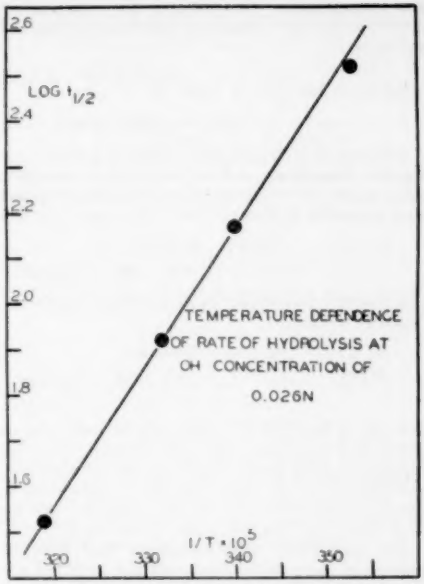

Fig. 6.—Temperature dependency of Reaction 1. Logarithm of half life in minutes plotted against the reciprocal of the absolute temperature of the run.

by substituting the conditions at 40°, i.e., $k_1 = (0.693/60)\ k'_1 = 0.011$ sec.$^{-1}$ and $\Delta H_a = 13{,}800$ calories. *s* comes out to be approximately 4.0×10^7 sec.$^{-1}$, or

$$k_1 = 4.0 \times 10^7\, e^{-13{,}800/RT} \text{ sec.}^{-1}$$
$$k'_1 = 3.5 \times 10^9\, e^{-13{,}800/RT} \text{ min.}^{-1} \qquad (11)$$

Determination of Temperature Dependency of Reaction 2.—It is evident from Fig. 6 that below *p*H of about 8, the over-all reaction is essentially that of Reaction 2, represented kinetically by Eq. (10). Direct evaluation of the temperature dependency of this reaction was rendered difficult by the unavailability of a suitable buffer which would maintain constant low hydroxyl ion concentration relatively independent of solution temperature. Equation (10), however, can be expressed also in terms of hydrogen

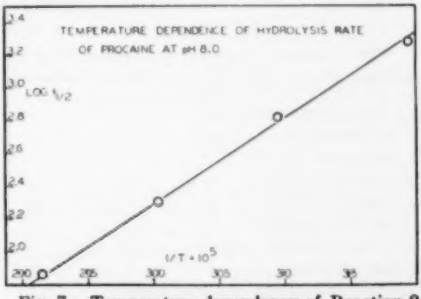

Fig. 7.—Temperature dependency of Reaction 2. Logarithm of half life in minutes plotted against the reciprocal of the absolute temperature of the run.

ion concentration by utilizing the relationship $(OH^-) = k_w/(H^+)$, where k_w is the ionic product of water, or

$$-\log t_{1/2} = \log \frac{k_w}{(H^+)} + \log k'_2 = pH + \log k_w + \log k'_2 \quad (12)$$

The heat of activation of k'_2, which is a measure of thermal dependency of Reaction 2, can be obtained from above by differentiating it at constant pH with respect to $1/T$, or

$$-\frac{d \log t_{1/2}}{d(1/T)} = \frac{d \log k_w}{d(1/T)} + \frac{d \log k'_2}{d(1/T)pH = \text{constant}}$$

and multiply through by 2.303 R, where R is the gas constant, we get

$$-\frac{Rd \ln t_{1/2}}{d(1/T)} = \frac{Rd \ln k_w}{d(1/T)} + \frac{Rd \ln k'_2}{d(1/T)pH = \text{constant}} \quad (13)$$

But $(Rd\ ln\ k_w)/[d1/T]$ is the heat of ionization of water into hydrogen and hydroxyl ions, approximately equal to 12.0 kilocalories per mole at 40°, and $(Rd\ ln\ k'_2)/[d(1/T)]$ is the heat of activation of Reaction 2, the value which we are seeking. All we need to do then is to determine the first term.

The logarithms of experimental half lives in minutes of procaine solutions buffered to pH of 8.0 are plotted in Fig. 7 as a function of $1/T$. Phosphate buffer was used in this study, the pH of this buffer being essentially independent of temperature in the range investigated (40° to 82°). A pH drop of roughly 0.25 unit was noted whenever the reaction was allowed to run nearly to completion. However, since the half-life determinations were made by analysis of the initial phase of the reaction during which no appreciable changes in the pH had occurred, the data are presumed to represent the rates at essentially pH of 8.0. The $-(Rd\ ln\ t_{1/2})/[d(1/T)]$ value calculated from the slope of the curve is 24 kilocalories. Or

$$\textit{heat of activation for Reaction 2} = \frac{Rd \ln k'_2}{d(1/T)} = 24 - 12 = 12 \text{ kilocalories per mole at } 40°.$$

The calculated value for the heat of activation (12 kilocalories) appear to be somewhat lower than that for most aromatic esters. For example, Ingold and Nathan (3) found 20.0 kilocalories per mole for the energy of activation for saponification of ethyl p-aminobenzoate. Their determination was, however, made in aqueous alcoholic solutions which usually lead to activation energies several kilocalories higher than those in water alone. Furthermore the positive charge on the alcohol nitrogen of procaine would tend to decrease the energy requirement for the formation of the activated complex with the negatively charged hydroxyl ion.

The corresponding frequency factor for Reaction 2, calculated for 40° is 7.4×10^8 sec.$^{-1}$. Or

$$k_2 = 7.4 \times 10^8\, e^{-12{,}000/RT} \text{ sec.}^{-1}$$

and

$$k'_2 = 6.4 \times 10^{10}\, e^{-12{,}000/RT} \text{ min.}^{-1} \quad (14)$$

Equation (14) can be substituted into Eq. (12) to give a rather simple relationship between half life of any buffered procaine solution at pH below 8.0 as a function of its pH and temperature,

$$-\log t_{1/2} = pH - pk_w - 10.8 - 2620/T$$

More general relationship can be obtained by substituting both 14 and 11 into 8, i.e.,

$$-\log t_{1/2} = \log(OH^-) + \log[1.6 \times 10^{-5} + (OH^-)] + \log[3.5 \times 10^9\, e^{-13{,}800/RT} + 1.0 \times 10^6\, e^{-13{,}000/RT}]$$

Because of the number of variables involved, it is felt that both of the above expressions are valid to only 0.1 logarithmic unit. This accuracy, however, is usually sufficient since it corresponds to the usual accuracy of a pH determination.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

α,α′-Dihydroxymethyl ether
2,5-Dinitrophenol
3,5-Dinitrophenol
Heptane-1,3-dithiol
Heptane-2,4-dithiol
2-Iodo-3-methylbutane
1,1-Diiodo-2-methyl propane
Benzoguanamine
2-(*p*-Acetaminostyryl)-6-dimethylamino quinolinium methochloride
5-Allyl-5-(1-methylbutyl) barbiturate
2-Amino-4-methylamino-6-ethylamino-1,3,5-triazine
Betaine aldehyde
Ethoxyethyl mercuric chloride
Isopropylmethyl mercuric chloride
Zinc isopropyl xanthate
Solochrome cyanine
1-Methyl-2-mercaptoimidazole
Quercetagetin
Oxyhemoglobin
Soya lipase

Heat Sterilization of Thermally Labile Solutions*,†

By T. HIGUCHI and L. W. BUSSE

A general criterion for choosing high or low temperature for sterilizing heat-sensitive pharmaceutical preparations is presented. A mathematical analysis of the competing processes involved shows that the choice of temperature is largely dependent on the relative magnitudes of the heats of activation of the sterilization and the drug deterioration reactions. Results indicate, for example, that for procaine solutions, autoclaving at 120° is preferable to prolonged sterilization at 100°.

THE PROBLEM of the relative merits of sterilizing heat-sensitive solutions at one or another temperature is often encountered by those engaged in pharmaceutical investigations. The question, for example, whether a procaine solution should be sterilized by autoclaving or sterilized at 100° has often been debated. In the present study, a general criterion for deciding whether higher or lower sterilization temperature is to be preferred in preparation of a thermally sensitive solution is presented. Although the actual application is made to the case of procaine solutions, the method is generally applicable to nearly all thermally labile solutions.

THEORETICAL CONSIDERATION

The sterilization process involves, essentially, irreversible denaturation of certain vital enzyme-proteins in the microorganisms present. Since inactivation of enzymes and denaturation of proteins appear to be of the unimolecular type, we can reasonably expect the death rate of microorganisms to follow the first order reaction law (1). In case of ordinary sterilization, however, the picture is complicated by the fact that several varieties of microorganisms are present. Furthermore, thermal sensitivity of a given type of an organism may be appreciably different at different stages of its life cycle. Nevertheless it has been found experimentally that the first order law is followed reasonably well during the terminal part of the sterilization process. This can be rationalized in view of the fact that only the most thermophilic of the original flora is present during the terminal course of the process.

On the basis of the above discussion and experimental evidence, we can write the following equation relating the death time, t_d, necessary for total sterilization to the absolute temperature of sterilization,

$$\ln t_d = \Delta H_s^*/RT + K \qquad (1)$$

where

t_d = the necessary sterilization time
ΔH_s^* = the heat of activation characteristic of killing most thermally resistant species present
R = the universal gas constant
T = the absolute temperature of sterilization
K = a constant depending on the number and kind of the most thermally resistant species present

The above expression is a modified form of the classical Arrhenius equation.

In Fig. 1 a typical relationship between ln t_d and temperature is shown. Also shown in the figure is the type of relationship found between the half life (the time required for 50% decomposition) of a thermally labile drug, such as procaine, and absolute temperature. This can be expressed similarly mathematically by the equation

$$\ln t_{1/2} = \Delta H_d^*/RT + C \qquad (2)$$

where

$t_{1/2}$ = the half life of the drug at temperature T
ΔH_d^* = the heat of activation of the decomposition reaction
C = a constant

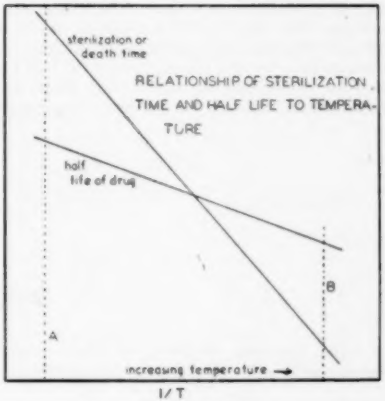

Figure 1

It is evident from the curve that at a low temperature (A) the half life of the drug may be much shorter than the necessary sterilization time; yet at a higher temperature (B) sterilization of the drug may be achieved in a small fraction of its half life. The determining factor for such a situation is that the sterilization line has a much sharper slope than the line representing the decomposition reaction.

* Received May 16, 1950, from the Research Laboratories of the School of Pharmacy, University of Wisconsin, Madison.
† This project was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

Since the relative slopes of these lines depend directly on the magnitude of their respective ΔH^* values, the above condition would be fulfilled if $\Delta H_s^* > \Delta H_d^*$. If we subtract Eq. 2 from Eq. 1 we obtain

$$\ln t_d - \ln t_{1/2} = [\Delta H_s^* - \Delta H_d^*] \frac{1}{RT} + K - C$$

or

$$\ln \frac{t_d}{t_{1/2}} = \frac{\Delta H_s^* - \Delta H_d^*}{R} \frac{1}{T} + \text{Constant}$$

$$\log \frac{t_d}{t_{1/2}} = \frac{\Delta H_s^* - \Delta H_d^*}{2.303 \times 1.987} \frac{1}{T} + \text{Constant}$$

and if we let $R = t_d/t_{1/2}$ then at temperatures T_A and T_B we have

$$\log \frac{R_A}{R_B} = \frac{\Delta H_s^* - \Delta H_d^*}{2.303 \times 1.987} \left[\frac{1}{T_A} - \frac{1}{T_B}\right] \quad (3)$$

The ratio R_A/R_B may be thought of as an improvement factor which expresses numerically the relative rate advantage of sterilization over decomposition in going from T_A to T_B. If R_A/R_B is greater than unity higher temperature is to be preferred. The value of R_A/R_B can be solved for any particular situation if numerical values of ΔH_s^* and ΔH_d^* are known.

ΔH_s^* values obtained for most thermophilic microorganisms and spores appear to be over 50 kilocalories. Those forms which have ΔH_s^* less than 50 kilocalories are relatively heat sensitive and do not influence the sterilization time. ΔH_d^* must be obtained for each drug.

Application to Procaine Solutions

Recently Higuchi, Havinga, and Busse (2) completed a chemical kinetic study of the rate of hydrolysis of procaine in solution. The data obtained indicate that the rate of hydrolysis of the ionic form of the drug was entirely different from that of the free base form. The calculated heat of activation for the hydrolysis of the salt form was 12 kilocalories. That for the free base form was shown to be 14 kilocalories. Since for all practical pharmaceutical preparations the compound is found in its salt form, only the former value need be considered. These workers also found that the hydrolysis reaction of ionic procaine was first order with respect to hydroxyl ions.

It appears from arguments put forth in the preceding section that since 12 kilocalories is much less than 50 kilocalories, sterilization of procaine solutions is more advantageously carried out at higher than at lower temperatures. In solutions buffered with acid type buffers, e.g., phosphate, borate, etc., the advantage of high temperature sterilization is, however, considerably less. This is due to the fact that although these buffers maintain fairly constant pH values over wide temperature ranges, hydroxyl ion concentration changes very rapidly with temperature. The increased hydrolysis rates resulting from higher hydroxyl concentration at higher temperatures correspond to added heat of activation of 10–13 kilocalories $\left(\text{the heat of ionization of water} = 2.30R\frac{dpk_w}{d(1/T)}\right)$ the exact value depending on temperature. In unbuffered or solutions buffered with an amine type buffer, the hydroxyl ion concentration is roughly independent of temperature and the correction may be omitted. In either case the apparent heat of activation of the hydrolysis reaction is still well below the previously mentioned 50 kilocalories value for the sterilization reaction. The calculated R_{100}/R_{120} for unbuffered procaine solutions is roughly *12*. For solutions buffered with acid type buffers R_{100}/R_{120}, calculated with Eq. 3, is approximately 8. For reasons stated above, sterilization by autoclaving for a short time is thus preferable to relatively long sterilization at 100° for procaine solutions.

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WHO MAKES IT?

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2-(*d*-Pantoylamino)ethylsulfono-4-chloroanilide
m-Hydroxypropadrine
bis-Dihydrocitronellyl ether
Dodecyltriethylammonium bromide
β-Fluoropropionic acid
Soya lipase
Carnegine
Estriol glucuronate
Barbatinic acid
Hemipic acid
Erythropleine
Xanthotoxin
Laurone

Comparison of the Effects of Injection of Pyrogenic Solutions by Intravenous and Intramuscular Routes*

By WILLIAM D. HARKNESS and BERT J. VOS

Rabbits were injected either intravenously or intramuscularly with a pyrogenic solution prepared from *Pseudomonas aeruginosa*, and their rectal temperatures were observed at hourly intervals for three and five hours, respectively. The rise in temperature following intramuscular injection was more gradual than that following intravenous injection and for a given dosage was considerably lower. Within narrow limits a linear relationship appears to exist between log dose of pyrogen and the maximum rise in temperature observed during the experimental period.

IN PREVIOUS investigations of pyrogenic materials (1–13) attention has centered chiefly on the effects which follow intravenous injection of such substances. The U. S. P. assay for pyrogen (14) is based on this type of reaction, and is generally believed to afford adequate protection in the case of fluids administered intravenously in large volume. The U. S. P. XII (15) contained the first official pyrogen assay and in slightly modified form that assay constitutes the present official method. Some of the collaborative work leading to the approval of these methods was described by Welch and co-workers (4) and McClosky and co-workers (6) in 1943.

On occasion the question of thermogenic effects of intramuscular injection of pyrogenic solutions has arisen. Rises in temperature have been observed in some instances following intramuscular injections in rabbits but no definite information as to the ratio of the intramuscular to the intravenous response has been available. It is the purpose of the present paper to describe the results of intravenous and intramuscular injection of solutions of comparable pyrogen content.

MATERIALS AND METHODS

In order to assure uniform pyrogenicity of the experimental solutions, pyrogenic material was prepared from *Pseudomonas aeruginosa* in accordance with the description of Welch and co-workers (4). Sufficient material was prepared to complete the experiment using one lot of the stock pyrogen solution and aseptic technique was employed in removal of portions of this sterile solution for the various parts of the work. Dilutions of the stock pyrogen solution were made with pyrogen-free isotonic sodium chloride solution such that the desired dosages were contained in a constant volume of 10 cc./Kg. for all intravenous injections and 0.333 cc./Kg. for all intramuscular injections.

All assays in which intravenous injection was employed were carried out in accordance with the official pyrogen method to the extent that after taking normal temperatures, the solutions were injected into an ear vein of rabbits and the effect of the pyrogen observed by noting their rectal temperatures at hourly intervals for three hours subsequent to the injection. Since the highest temperature commonly occurred at the third hour, two additional readings were taken on the rabbits receiving the largest dose of pyrogens. In them the temperature fell during the fourth and fifth hours but was still an average of 1.3° above normal at the final reading. With the rabbits receiving intramuscular injections the hourly temperature readings were continued for five hours in each case. In most instances this was sufficient to include the peak of the temperature rise, but at one dose (0.005 cc./Kg.) there was an average rise of 0.1° in the fifth hour. Intramuscular injections were made deep into the biceps femoris with a $1^1/_4$-inch needle.

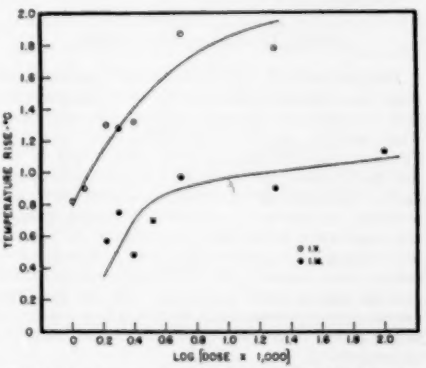

Fig. 1.—Curves showing the relationship between average temperature effects following intravenous and intramuscular administration of graded levels of stock pyrogen solution to rabbits.

The animals were restrained in stocks during the respective three or five hours of use, the stocks being of the type used in all routine pyrogen testing in this laboratory. These encircle the neck of the rabbit but permit some freedom of motion for the remainder of the body.

* Received January 12, 1950, from the Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.
A preliminary report was presented at the April, 1949, meeting of the Federation of American Societies for Experimental Biology.

Molitor and co-workers (7) reported that the use of the rectal thermometer in taking the repeated temperatures required in pyrogen assays was likely to produce fluctuations in rabbit temperature not observed when compared with temperature measurements obtained by the use of rectal thermocouples inserted for the duration of the assays. However, as these authors point out, the fluctuations are reduced after the animals, through repeated use, have become accustomed to the handling necessitated by the manual temperature technique. As a temperature recording device was not available at the time of this study, only animals which had been in use for pyrogen testing for at least several months were employed. The present work was carried out concurrently with routine regulatory testing and therefore it was felt that intramuscular doses of larger volume should be avoided because of the possibility of discomfort or harm to the permanent rabbit colony.

TABLE I.—AVERAGE MAXIMUM RISE IN RECTAL TEMPERATURE ABOVE STARTING TEMPERATURE OF RABBITS INJECTED INTRAVENOUSLY OR INTRAMUSCULARLY WITH GRADED LEVELS OF STOCK PYROGEN SOLUTION

Dose of Stock Pyrogen Solution, Cc./Kg.	Average Maximum Temperature Rise			
	Intravenous Injection, ° C.		Intramuscular Injection, ° C.	
0.00100	0.82	±0.14[a]	...	...
0.00125	0.90	±0.24	...	...
0.00167	1.30	±0.26	0.57	±0.12
0.00200	1.28	±0.08	0.75	±0.19
0.00250	1.32	±0.20	0.48	±0.13
0.00333	...	...	0.70	±0.08
0.0050	1.87	±0.13	0.97	±0.25
0.0200	1.78	±0.21	0.90	±0.12
0.100	...	...	1.13	±0.12

[a] Standard error of mean.

RESULTS AND DISCUSSION

For purposes of evaluation of the responses the maximum temperature increase over the preinjection temperature of each animal was employed. This would be at any of the three hourly readings for those injected intravenously and at any of the five hourly readings for those injected intramuscularly. The average of these maximum rises was obtained for each group of rabbits receiving doses of the respective concentrations as indicated in Table I. Six animals were used at each dose. Pyrogen-free normal saline injected intramuscularly resulted in an average rise of 0.33° indicating that the handling necessary for intramuscular injection is not a factor in the higher responses obtained following injection of pyrogen.

In U. S. P. XIII assays for pyrogen, 0.6° is considered a significant rise in temperature and all of the average rises resulting from intravenous administration shown in Table I are above this figure. For intramuscular doses it was decided that starting with dose 0.00167 cc. per Kg. and with higher dosages the average responses were definite enough to be considered. In fact with even the two lowest average temperature rises, 0.57 and 0.48°, three of six animals in each case responded with the significant rise in temperature, that is 0.6° or more.

When the temperature response is plotted against the logarithm of the dose (Fig. 1) a linear relationship is seen to exist within certain limits. The two curves start out fairly parallel but the one for intramuscular injection reaches a plateau much sooner. At any given dose the intravenous route produces a higher rise in temperature. The graded response to intravenous injection of pyrogen has been previously observed by a number of investigators but was repeated in this work in order to provide a basis for comparison with the results of intramuscular injection. The curve indicates that there is a graded response following the intramuscular injection of increasing doses of pyrogen in rabbits.

SUMMARY AND CONCLUSIONS

1. Rises in temperature were observed in rabbits injected intramuscularly with pyrogenic solutions of known concentration, prepared from *Pseudomonas aeruginosa*.

2. These rises in temperature were lower than those obtained on intravenous injection of comparable quantities of pyrogen.

3. The responses to intramuscular administration of pyrogen were found to be graded, depending on dose administered. This has been known to be the case with intravenous injection when average rises of groups of rabbits receiving varying doses are compared.

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Electrometric Titration of Volatile Oils and Volatile Oil Isolates Using Lithium Aluminum Hydride*,†

By CARL J. LINTNER,‡ JOSEPH V. SWINTOSKY, DONALD ZUCK,§ and T. HIGUCHI

The development of an electrometric lithium aluminum hydride titration procedure for the quantitative determination of certain functional groups has been described in a previous paper. In this report results of an investigation studying the feasibility of this method of analysis as applied to essential oils and essential oil isolates are presented. Analytical data were obtained for a number of substances of pharmaceutical interest. The value of the method as a criterion for purity and identity of volatile oils is also discussed.

THE ANALYSIS of volatile oils and related substances is a highly specialized field of analytical chemistry which requires diverse physical and chemical procedures. The analytical procedures are employed to characterize oils, to detect spoilage, to detect adulteration and sophistication and for the general purpose of establishing the identity and validity of these products. Such useful quantitative indices as optical rotation, refractive index, acid number, ester number, etc., which collectively aid in establishing the purity and identity of these substances are usually obtained. The present investigation was undertaken with the supposition that a new useful quantitative index may be obtainable for volatile oils and related substances by application of the new quantitative electrometric titration method employing lithium aluminum hydride. The purpose of the initial study was to establish the feasibility of this type of analysis for volatile oils.

The application of the lithium aluminum hydride electrometric titration procedure (HLS method) to the quantitative determination of various functional groups has been described previously (1–3), and allusion has already been made to the possibility of using this procedure in the analysis of volatile oils. It is observed, in the limited amount of data resulting from the use of the HLS method, that alcohols, phenols, aldehydes, ketones, esters, and carboxylic acids, compounds which are present in essential oils, react quantitatively with lithium aluminum hydride in the following manner:

Alcohols and Phenols

$$4ROH + LiAlH_4 \rightarrow LiAl(OR)_4 + 4H_2$$

Aldehydes

$$4RCHO + LiAlH_4 \rightarrow (RCH_2O)_4LiAl$$

Ketones

$$4R_2CO + LiAlH_4 \rightarrow (R_2CHO)_4LiAl$$

Esters

$$2RCOOR' + LiAlH_4 \rightarrow {}^1/_2(RCH_2O)_4LiAl + {}^1/_2(R'O)_4LiAl$$

Carboxylic Acids

$$4RCOOH + 3LiAlH_4 \rightarrow (RCH_2O)_4LiAl + 2LiAlO_2 + 4H_2$$

Lithium aluminum hydride does not as a rule react with ether linkages or ethylenic double bonds. In substances such as cinnamic acid, where the unsaturated bond is definitely activated by conjugation with an aromatic nucleus on one side and a readily reducible group on the other, reduction of the unsaturated linkage is observed.

It is common knowledge that volatile oils consist of mixtures of the type compounds enumerated above together with other types which do not react with lithium aluminum hydride. Volatile oils then should undergo reaction with this agent; and if different lots of a given volatile oil are qualitatively and quantitatively similar (the premise upon which all present indices are based) then a lithium aluminum hydride equivalence of rather constant value for individual oils should be establishable.

APPARATUS AND PROCEDURE

The description of the apparatus and the preparation of the standard reagents for this procedure have been fully described (2) in previous publications. Techniques consistent with the established procedure were followed in these analyses.

Exactly 50 ml. of purified tetrahydrofuran (THF) and exactly 5 ml., or 10 ml., of THF solution of lithium aluminum hydride were pipetted into the reaction vessel. The weighed volatile oil or isolate was added to this solution and allowed to react for fifteen minutes under magnetic stirrer agitation. A standard solution of 5% *n*-propyl alcohol by volume in benzene was then used in the potentiometric titration of excess lithium aluminum hydride. For each determination a blank run was conducted with

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the identical procedure except for the addition of the sample. The equivalence of each substance was expressed in milliequivalents per gram of sample. The following substances were analyzed:

Eucalyptol.—Mallinckrodt U. S. P. grade eucalyptol was analyzed after drying by distillation over metallic sodium. The refractive index (n) of this purified substance at 25° was 1.4498.

Methyl Salicylate.—Merck methyl salicylate was dried by distillation over metallic sodium (n_{25} = 1.5341) and analyzed.

Eugenol.—Merck eugenol dried over Drierite for forty-eight hours (n_{25} = 1.5375) was analyzed without further treatment.

Benzaldehyde.—Merck U. S. P. grade benzaldehyde was dried by distilling over metallic sodium (n_{25} = 1.5420) before analysis.

Thymol.—Magnus, Mabee, and Reynard U. S. P. grade thymol was dried on a porous plate (m. p. 51–52°) and analyzed without further pretreatment.

Menthol.—Merck U. S. P. grade menthol was dried on a porous plate (m. p. 43–44°) and analyzed without further pretreatment.

Borneol.—A student preparation derived from hemlock oil was recrystallized three times from benzene (m. p. = 208–210°) and analyzed without further treatment.

Citronellol.—City Chemical Corporation of New York citronellol was dried by standing over magnesium perchlorate for forty-eight hours (n_{25} = 1.4500) and analyzed.

Terpineol.—Fritzsche Bros. terpineol was dried by standing over magnesium perchlorate for forty-eight hours (n_{25} = 1.4798) and analyzed without further treatment.

***d*-Carvone.**—A laboratory-prepared sample[1] of *d*-carvone was dried by standing over magnesium perchlorate for forty-eight hours (n_{25}=1.4969) and analyzed.

Piperonal (Heliotropin).—Fritzsche Bros. piperonal was dried on a porous plate (m. p. = 37 to 37.5°) and subjected to analysis.

***l*-Methone.**—Eastman Kodak *l*-menthone was dried by standing over Drierite for forty-eight hours (n_{25} = 1.4475) and analyzed without further treatment.

[1] Samples furnished by Dr. Gordon Swoboda, now at the School of Pharmacy, University of Kansas.

Linaloöl.—Fritzsche Bros. linaloöl was dried by standing over magnesium perchlorate for forty-eight hours (n_{25} = 1.4689) and analyzed.

Limonene.—Fritzsche Bros. limonene was dried by standing over magnesium perchlorate for forty-eight hours (n_{25} = 1.6727) and analyzed.

The following volatile oils were each dried over C. P. calcium chloride for forty-eight hours before analysis:

1. Selected Peppermint Oil. Fritzsche Bros.
2. Spearmint Oil. Fritzsche Bros. U. S. P. grade.
3. Rosemary Oil. Fritzsche Bros. U. S. P. grade.
4. Lavender Oil. Fritzsche Bros. U. S. P. grade.
5. Cinnamon Ceylon Imitation Oil. Fritzsche Bros.
6. Clove Oil. Magnus, Mabee, and Reynard U. S. P. grade.
7. Lemon Oil, California Coldpressed. Fritzsche Bros. U. S. P. grade.
8. Thyme Oil, White Extra Fine. Fritzsche Bros.
9. Bergamot F. B. Oil, Extra Fine. Fritzsche Bros.
10. Litsea Guatemalensis Oil[1].

RESULTS AND DISCUSSION

The results of the analyses on the various volatile oils and related substances are given in Tables I and II. The equivalents per sample are expressed as the fraction of a mole of alcohol to which a given sample is equivalent. The milliequivalent per gram is then readily calculated from the expression:

$$\text{M. E. per gram} = \frac{\text{Equivalents per sample} \times 1000}{\text{Weight of sample}}$$

The data on the volatile oil isolates show that for each substance titrated, experimental and theoretical values check reasonably well except for borneol. The method is thus quantitative and a determination of the M. E. per gram on a mixture of such substances as they may occur in volatile oils should serve as a useful index in ascertaining the relative amounts of reactive oxygenated components in a natural product of this type.

TABLE I.—LITHIUM ALUMINUM HYDRIDE EQUIVALENCE VALUES FOR SOME VOLATILE OIL ISOLATES

Substance Analyzed	Sample Weight, Gm.	$LiAlH_4$ Solution, Cc.	Vol. Std. Alc. Soln. Consumed		Theoretical Equivalents Per Sample	Experimental Equivalents Per Sample	Equivalents Per Mole	M. E. Per Gm.
			Blank	With Sample				
Eucalyptol	0.4815	5.00	7.20	7.20	0	0.0000	0.00	0.00
Me. Salicylate	0.2968	10.00	14.62	5.85	0.00583	0.005870	3.009	19.77
Eugenol	0.3873	5.00	6.40	2.91	0.002365	0.002336	0.987	6.01
Benzaldehyde	0.4831	10.00	14.71	7.87	0.004552	0.004578	1.005	9.47
Thymol	0.3002	10.00	17.80	14.70	0.001998	0.002074	1.038	6.91
Menthol	0.4927	10.00	15.77	10.93	0.003153	0.003239	1.027	6.57
Borneol	0.3052	5.00	5.77	2.55	0.001979	0.002155	1.089	7.06
Citronellol	0.9048	10.00	17.55	9.00	0.005790	0.005722	0.988	6.32
Terpineol	0.5011	10.00	17.55	12.68	0.003249	0.003260	1.004	6.51
d-Carvone	0.4682	10.00	17.35	12.65	0.003117	0.003146	1.009	6.72
Piperonal	0.7365	10.00	17.10	9.80	0.004906	0.004886	0.996	6.63
l-Menthone	0.6108	10.00	17.10	10.95	0.003960	0.004116	1.038	6.73
Linaloöl	0.9420	10.00	17.35	8.10	0.006107	0.006191	1.013	6.57
Limonene	0.9258	10.00	17.35	17.29	0	0.000040	0	0

TABLE II.—ANALYTICAL RESULTS FOR SOME COMMON VOLATILE OILS

Name	Sample Weight, Gm.	$LiAlH_4$ Solution, Ml.	Vol. Std. Alc. Blank, Ml.	Soln. Consumed with Sample, Ml.	Equivalents Found	M. E. Per Gm.
Spearmint Oil	0.5357	10.00	16.50	8.55	0.005321	9.93
	0.5182	10.00	16.50	8.80	0.005154	9.94
Rosemary Oil	0.5390	10.00	16.50	9.50	0.004685	8.69
	0.4816	10.00	17.94	11.70	0.004176	8.67
Thyme Oil	0.5102	10.00	16.50	8.55	0.005321	10.43
	0.5267	10.00	16.50	8.10	0.005622	10.67
Peppermint Oil	0.5164	10.00	13.35	4.25	0.006091	11.79
	0.4112	10.00	17.94	10.68	0.004859	11.82
Lemon Oil	0.4281	5.00	3.95	3.51	0.002945	0.688
	0.4887	5.00	3.95	3.45	0.003347	0.685
Orange Oil	0.7182	10.00	20.33	19.15	0.0007898	1.09
	0.3463	5.00	3.95	3.38	0.0003815	1.09
Clove Oil	0.5433	5.00	3.95	2.225	0.001138	2.09
	0.3991	5.00	4.70	3.48	0.0008165	2.05
Bergamot Oil	0.4034	5.00	3.30	1.72	0.001058	2.62
	0.4401	5.00	3.30	1.55	0.001171	2.66
Lavender Oil	0.4213	5.00	3.30	0.62	0.001794	4.26
	0.4195	5.00	3.30	0.70	0.001740	4.15
Cinnamon Oil	0.5060	5.00	9.90	3.95	0.003982	7.86
	0.4897	10.00	17.94	12.24	0.003815	7.79
Litsea Guatemalensis Mez. Oil	0.5931	10.00	17.35	8.90	0.005656	9.53
	0.6744	10.00	17.35	7.60	0.006526	9.67

TABLE III.—ANALYSIS OF SYNTHETIC MIXTURES OF VOLATILE OILS

Mixture	Actual Lemon Oil, %	Actual Spearmint Oil, %	Experimental Lemon Oil %	Experimental Spearmint Oil, %
I	71.58	28.42	71.8	28.2
II	53.26	46.74	52.8	47.1
III	35.58	64.42	35.8	64.2
IV	20.26	79.74	19.6	80.4

The results of the analyses on the volatile oils of Table II indicate the reproducibility of the HLS method, and the possibilities for this procedure in establishing a lithium aluminum hydride equivalence as a quantitative index for purity and identity of these products. The low M. E. per gram in lemon oil and orange oil, for example, is in keeping with the fact that each of these oils contain approximately 90% or more of limonene plus other hydrocarbons which do not react with lithium aluminum hydride. The relatively high M. E. per gram for such oils as spearmint, thyme, and peppermint likewise is in keeping with our knowledge that these oils contain high percentages of esters, phenols, alcohols, and other components which react with lithium aluminum hydride. The data for all of the oils correlate well with our present knowledge of the qualitative and quantitative chemical composition of these products. The HLS method thus provides a simple, readily reproducible procedure for a rapid determination of a chemical index which should be a useful tool in the analysis and identification of volatile oils.

The analytical possibilities of the method are further illustrated in Table III, where results of analysis of several synthetic mixtures of lemon oil and spearmint oil are shown. It is evident that results are reasonably quantitative in nature even at the present stage of development of the method.

SUMMARY

The application of the HLS method of potentiometric titration with lithium aluminum hydride has been illustrated in analyses of volatile oils and volatile oil isolates. The titration of known volatile oil isolates gives experimental values which check well with theoretical values. Titration values on volatile oils correlate well with predicted values based on knowledge of the chemical compositions of these oils. The possible usefulness of the lithium aluminum hydride equivalence as an index for purity and identity of volatile oils has been pointed out.

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Application of Lithium Aluminum Hydride Titrimetric Method to Pharmaceutical Compounds*,†

By CARL J. LINTNER,‡ DONALD A. ZUCK,§ and T. HIGUCHI

The feasibility of lithium aluminum hydride titrimetric method for analysis of various pharmaceutical type compounds was investigated. Nineteen different substances were analyzed with an average error of less than two per cent. Compounds studied included amines, acids, lactones, alkaloids, alcohols, aldehydes, esters, and phenols. In all cases the reaction with the hydride followed the predicted stoichiometric relationship.

IN INVESTIGATIONS of either crude or pure pharmaceuticals it is often desirable to know the concentration of functional groups present. Such information is of value not only for purely analytical reasons but is also often necessary for characterization and identification purposes. It is evident that knowledge, for example, of milliequivalences of aldehydic or ketonic grouping present in an unknown sample would prove very useful in many cases.

Such information can be readily obtained for acidic or alkaline groups by the usual alkalimetric or acidimetric procedures. Comparable information for alcoholic, aldehydic, ketonic, and many other groups is not, however, easily obtainable by the present methods. In this paper, results of application of a previously described method (1, 2) which utilizes lithium aluminum hydride solution for volumetric determination of these latter functional groups are presented.

THEORY

The theoretical basis of the method has been covered in detail in previous papers (1, 2). The method is essentially based on quantitative reactions of lithium aluminum hydride with various functional groups. These are:

Alcohols, glycols, and phenols

$$4ROH + LiAlH_4 \rightarrow LiAl(OR)_4 + 4H_2$$

Carboxylic acids

$$4RCOOH + 3LiAlH_4 \rightarrow (RCH_2O)_4LiAl + 2LiAlO_2 + 4H_2$$

Primary amines

$$2RNH_2 + LiAlH_4 \rightarrow LiAl(RN)_2 + 4H_2$$

Secondary amines

$$4R_2NH + LiAlH_4 \rightarrow LiAl(R_2N)_4 + 4H_2$$

Aldehydes

$$4RCHO + LiAlH_4 \rightarrow (RCH_2O)_4LiAl$$

Ketones

$$4R_2CO + LiAlH_4 \rightarrow (R_2CHO)_4LiAl$$

Esters

$$2RCOOR' + LiAlH_4 \rightarrow {}^1/_2(RCH_2O)_4LiAl + {}^1/_2(R'O)_4LiAl$$

Anhydrides

$$(RCO)_2O + LiAlH_4 \rightarrow {}^1/_2(RCH_2O)_4LiAl + {}^1/_2LiAlO_2$$

Acid chlorides

$$2RCOCl + LiAlH_4 \rightarrow {}^1/_2(RCH_2O)_4LiAl + {}^1/_2AlCl_3 + {}^1/_2LiCl$$

Amides

$$2RCONR_2 + LiAlH_4 \rightarrow 2RCH_2NR_2 + LiAlO_2$$

$$4RCONR_4' + 2LiAlH_4 \rightarrow LiAl(OCH_2R)_4 + LiAl(NR_2')_4$$

$$4RCONHR' + 3LiAlH_4 \rightarrow LiAl(OCH_2R)_4 + 2LiAl(R'N)_2 + 4H_2$$

Nitriles

$$2RCN + LiAlH_4 \rightarrow (RCH_2N)_2LiAl$$

Aromatic nitro compounds

$$2RNO_2 + 2LiAlH_4 \rightarrow R{-}N{=}N{-}R + 4H_2 + 2LiAlO_2$$

In addition to the organic compounds listed above, lithium aluminum hydride reacts with the following inorganic compounds:

Water

$$LiAlH_4 + 4H_2O \rightarrow LiOH + Al(OH)_3 + 4H_2$$

Ammonia

$$2LiAlH_4 + 5NH_3 \rightarrow [LiAlH(NH_2)_2]_2NH + 6H_2$$

Metal halides

$$4MCl_n + nLiAlH_4 \rightarrow 4MH_n + nLiCl + nAlCl_3$$

Carbon dioxide

$$4CO_2 + 3LiAlH_4 \rightarrow 3LiAl(OCH_3)_4 + 2LiAlO_2$$

Two different reactions may take place with carbon dioxide. With an excess of carbon dioxide, derivatives of formaldehyde are formed, while with an excess of the hydride, methanol derivatives are produced. The latter reaction has been investigated for the preparation of methanol containing isotopic carbon.

From available evidence most reactions are quantitative with very little side reactions.

The essential difference between the present method and the lithium aluminum hydride methods (3–5) that have been previously proposed is in the analysis of lithium aluminum hydride which had undergone reaction. Earlier methods have been based on rather complicated gasometric determinations; in the method of Higuchi, Lintner, and

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Schleif, use is made of electrometric analysis of the residual lithium aluminum hydride.

The basis of the electrometric method lies in the fact that hydrides are powerful reducing agents. On addition of lithium aluminum hydride solution to a solution containing alcohol for example, all hydride would be destroyed until all the alcohol had reacted. As soon as a slight excess of hydride is present the reduction potential of the system (as indicated, for example, by a bright platinum or silver electrode) should increase markedly.

PLAN OF WORK

In the present study an investigation of the feasibility of the method of Higuchi, Lintner, and Schleif for analysis of various typical pharmaceutical compounds has been made. The methods used and results obtained are reported in the following sections.

APPARATUS

The apparatus used in this series has already been described in a previous paper (2). It is shown schematically in Fig. 1. It is essentially an electrometric titration assembly with an isolated silver electrode as the reference electrode and a bright silver electrode as the indicator electrode. Because of the unusual nature of the electrolyte used, it is more desirable to connect the reference instead of the usual indicator electrode to the high resistance lead of the indicating voltmeter. Beckman Model H pH meter has been found to be satisfactory for the latter function.

REAGENTS

Reagents used were prepared in the following manner:

a. Tetrahydrofuran, which is used as the solvent for the lithium aluminum hydride and also in the electrolytic system, was purified by double distillation over lithium aluminum hydride.

b. Lithium aluminum hydride solution was prepared by refluxing an excess of the compound with purified tetrahydrofuran. This was allowed to stand overnight and then centrifuged. The clear supernatant solution was poured off and stored in a rubber-stoppered bottle.

c. Standard alcohol-benzene solution was made by adding dry, thiophene-free benzene to dry, analytical grade, normal propyl alcohol. Exactly 50 ml. of the alcohol was made up to one liter in a volumetric flask. The solution was stored in an automatic burette fitted with a Drierite-Ascarite tube.

Samples analyzed were first treated in the following fashions:

Aniline—Mallinckrodt, $n^{26} = 1.5797$ dried by distilling from metallic sodium.

Diphenylamine—M. p. 52 to 53° dried by rubbing on a porous plate.

α-Naphthylamine—M. p. 48 to 49.5° dried by rubbing on a porous plate.

Dimethylaniline—Baker Chemical Works, $n^{25} = 1.5550$ was dried by distillation.

Benzoic Acid—Analytical grade, m. p. 121 to 121.5° dried by rubbing on a porous plate.

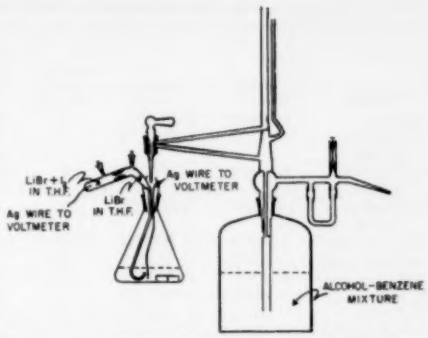

Fig. 1.—A schematic diagram of the apparatus used in the present study.

Salicylic Acid—Analytical grade, m. p. 158 to 159° dried by rubbing on a porous plate.

Amidopyrine—M. p. 107 to 109° dried in hot air oven at 90° for forty-eight hours.

Coumarin—Fritsche Brothers, m. p. 68 to 69.5° dried by rubbing on a porous plate.

Morphine—Merck, recrystallized from an alcohol-water mixture, dried in hot air oven at 114° for forty-eight hours. M. p. 252.5 to 254°.

Cinchonidine—Lehn and Fink, recrystallized from a water-alcohol mixture and dried in oven at 114° for forty-six hours. M. p. 202.5 to 204°.

Verbenalol—Aglucone prepared from verbenalin a glucoside isolated from *Verbena stricta*. Courtesy of Dr. L. M. Parks. M. p. 128 to 130°.

Methyl Salicylate—Merck, $n^{26} = 1.5341$ dried by distillation.

Benzaldehyde—Merck, $n^{26} = 1.5420$ dried by distillation.

Vanillin—Fritsche Brothers, m. p. 81 to 82° dried by rubbing on a porous plate.

Aspirin—M. p. 135 to 136° dried in an oven at 102° for forty-eight hours.

Salol—M. p. 41.5 to 43° dried by rubbing on a porous plate.

Benzyl Alcohol—$n = 1.5368$ dried by distillation.

Thymol—Magnus and Mabree, m. p. 51 to 52° dried by rubbing on a porous plate.

Menthol—Merck, m. p. 43 to 44° dried by rubbing on a porous plate.

PROCEDURE

The lithium aluminum hydride solution was pipetted into a 125-ml. glass-stoppered Erlenmeyer flask. The dried compound to be analyzed was mixed with 25.0 ml. of previously dried tetrahydrofuran. The reaction mixture was then either heated under reflux for sixty minutes or, alternatively agitated with a mechanical stirrer for thirty minutes. In either case, the mixture was protected by a Drierite drying tube. The electrodes were then inserted and the solution titrated with the 5% n-propyl alcohol solution in benzene. The blank determination was made with the same procedure except for addition of the sample.

The gram equivalents of functional group present in the sample were calculated according to the following formula:

TABLE I.—ANALYTICAL RESULTS OBTAINED WITH SOME PHARMACEUTICAL COMPOUNDS

Sample	Sample Wt., Gm.	Moles Present	Method of Reacting	Vol. of $LiAlH_4$ Soln., Ml.	Standard Alcohol —Titration, Ml.— Blank	Sample	Gram Equivalents of Alcohol Consumed by Sample	Equivalent/Mole	Predicted Equivalence
Aniline	0.4843	0.005201	X	40.0	39.80	24.70	0.010110	1.94	2
Diphenylamine	0.4703	0.002779	X	10.0	12.85	8.50	0.002912	1.05	1
α-Naphthylamine	0.4821	0.003367	Y	20.0	27.31	17.31	0.006693	1.99	2
Dimethylaniline	0.6990	0.005768	Y	40.0	38.65	38.50			0
Benzoic acid	0.4963	0.004064	X	20.0	22.30	4.68	0.011793	2.90	3
Salicylic acid	0.5822	0.004215	X	15.0	27.31	2.09	0.16880	4.00	4
Amidopyrine	0.6364	0.002751	X	10.0	19.90	15.76	0.002771	1.01	1
Coumarin	0.4879	0.003338	X	10.0	19.90	5.24	0.009812	2.94	3
Morphine	0.5102	0.001789	X	10.0	18.75	13.45	0.003547	1.98	2
Cinchonidine	0.4931	0.001675	X	10.0	18.75	16.15	0.001740	1.04	1
Verbenalol	0.1125	0.0004977	X	5.0	7.68	3.94	0.002503	5.03	5
Methyl Salicylate	0.2968	0.001951	X	10.0	14.62	5.85	0.005870	3.01	3
Benzaldehyde	0.4831	0.004552	X	10.0	14.71	7.87	0.004578	1.00	1
Vanillin	0.2650	0.001742	X	10.0	19.42	14.17	0.003514	2.02	2
Aspirin	0.5078	0.002818	X	15.0	27.31	6.44	0.013968	4.96	5
Salol	0.5033	0.002349	X	10.0	17.73	7.25	0.007014	2.99	3
Benzyl alcohol	0.5016	0.004638	X	10.0	20.10	13.14	0.004658	1.00	1
Thymol	0.3002	0.001998	X	10.0	20.10	17.00	0.002075	1.04	1
Menthol	0.4927	0.003153	X	5.0	5.77	0.93	0.003239	1.03	1

X = Refluxed one hour. Y = Stirred thirty minutes.

$$\text{Gram equivalents content} = \frac{(B - V)M}{1000}$$

where

B = ml. of standard alcohol solution consumed by the blank
V = ml. of standard alcohol solution consumed by sample titration
M = molarity of the standard alcohol solution used

DISCUSSION AND CONCLUSIONS

The experimental values obtained by a previously described method agree favorably with the predicted value. The higher values are usually due to low molecular weight impurities. Water, in particular, is an important source of error. Lower values usually indicate an incomplete reaction as with aniline and benzoic acid.

Since the end point obtained by the method is extremely sharp and reproducibility is usually good under proper conditions (2), even better analytical accuracy appears to be possible with further refinement.

After completion of this work, it has been found that lithium aluminum hydride solutions react with molecular oxygen. For this reason improvement in precision can be obtained by running the analysis under nitrogen cover.

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Notes

A Note on the Preparation of Dipicolinic Acid*

By TAITO O. SOINE and MYRON R. BUCHDAHL

BECAUSE of a research program in this laboratory it was necessary to prepare relatively large amounts of dipicolinic acid (2,6-pyridine dicarboxylic acid). A survey of the literature indicated that the oxidation of 2,6-lutidine (2,6-dimethyl pyridine) by potassium permanganate (1, 2) would be the most practical method of preparation. From the standpoint of yield the method of Black and co-workers (2) seemed the most promising.

In several attempts to duplicate their procedure we were unable to obtain yields anywhere near those claimed. Furthermore, close personal attention was demanded from the operator for a long period of time and the apparatus had to be specially constructed. Their method called for a large excess of potassium permanganate over the theoretical amount required for the oxidation. The use of excess permanganate in our experience has resulted in decreased yields rather than increased yields as claimed. Using their procedure, an *apparent* yield corresponding to theirs was obtained by long cooling in the refrigerator, but the product was highly contaminated with potassium salts.

Strangely enough, the impure mixture gives an apparent melting point approximating that of hydrated dipicolinic acid (m. p. 235–237°). Among our products of oxidation we had isolated monopotassium dipicolinate (m. p. > 360°). When this was mixed in varying proportions with dipicolinic acid it was observed that the mixtures melted near the melting point of hydrated dipicolinic acid. This leads us to believe that the product of Black and coworkers may have been highly contaminated with potassium salts even though their melting point seemed correct.

Because of the above considerations a simple procedure has been developed which is believed to be more adaptable to the average laboratory and yields a hydrated dipicolinic acid uncontaminated with potassium salts. Anhydrous dipicolinic acid is readily obtainable by heating the product at 100° under reduced pressure. Over 30 oxidations have been performed by this procedure employing the theoretical amount of permanganate with yields varying from 40% (of the theoretical) to 50% using, respectively, 1.92 and 0.27 moles of 2,6-lutidine.

No attention is required by the operator during the oxidation and the apparatus consists of a simple reflux setup. Monopotassium dipicolinate is obtained as an intermediate and is converted to dipicolinic acid with hydrochloric acid.

EXPERIMENTAL

Fifty-three and one-half grams of 2,6-lutidine (0.5 mole, 57 cc.)[1] and 321 Gm. of potassium permanganate (2.03 moles) were added to 2800 cc. of water and refluxed in a 5-liter round-bottom flask for twenty-four to forty-eight hours on a steam bath. An extra long reflux condenser was employed and the flask was well covered with towels. Completion of the reaction was determined by dipping a strip of filter paper into the clear, colorless liquid over the well-settled manganese dioxide sludge.

The solution was filtered while hot through a Büchner funnel and the sludge washed with 6 to 9 liters of boiling water or until the washings were rendered neutral to litmus paper. The combined filtrate and washings were concentrated on a steam bath to a volume of 1500 to 2000 cc. The concentrated solution was then filtered and placed in the refrigerator to cool.

The cooled filtrate was acidified with concentrated hydrochloric acid to a *p*H of 1.5. This required approximately 120 cc. of the acid. At this point, monopotassium dipicolinate came down as a flocculent precipitate. The acidified mixture was placed in the refrigerator for fifteen to twenty minutes or until the precipitate began to settle. Upon filtering and drying, 59 Gm. of the monopotassium salt was obtained.

The monopotassium salt was dissolved by boiling in 800 cc. of 5% (*w/v*) hydrochloric acid. The acid solution, when cooled slowly to room temperature, yielded 43 Gm. of hydrated dipicolinic acid. Upon chilling the acid filtrate, 1.5 Gm. of anhydrous dipicolinic acid (m. p. 226°) was obtained.

The hydrated dipicolinic acid was recrystallized from 600 cc. of boiling water and upon cooling slowly to room temperature yielded 38.5 Gm. of potassium-free dipicolinic acid (m. p. 235–237°). On chilling the aqueous filtrate, 3 to 3.5 Gm. of potassium-free anhydrous acid was obtained.

The total yield was 43.73 Gm. of hydrated dipicolinic acid ($C_7H_5O_4N \cdot 1^1/_2H_2O$) and represented a 45% yield of the theoretical amount. The identity of the acid had been established earlier by carbon and hydrogen analyses and by conversion to the dichloride and dimethyl ester (3).

SUMMARY

1. A simple method for the preparation of dipicolinic acid employing potassium permanganate and 2,6-lutidine as the reactants has been described.

2. The method possesses several advantages over the procedures described in the literature.

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* Received February 23, 1950, from the College of Pharmacy, University of Minnesota, Minneapolis.

[1] 2,6-Lutidine is a product of Koppers Co., Inc., Pittsburgh, Pa., and is 96% pure.

A Note on the Use of Pigeons for the Assay of Digitalis Products*

By HAROLD H. FISHER and CLEMENS A. SUDOL

THE adoption by the U. S. P. XIII (1) of the pigeon test for digitalis and associated materials has presented a number of problems in our laboratory. The two most immediate problems, ease of cannulation and immobilization of the pigeons throughout the assay period, were investigated by us. Our solution to the above problems is being submitted as a possible aid to others who may encounter similar difficulties.

The difficulty of immobilizing the pigeons throughout the assay period was overcome as follows: Flat iron strips $1^1/_4$ in. x $10^1/_2$ in. long were arranged on a table top 25 in. x 48 in. as shown (Fig. 1).

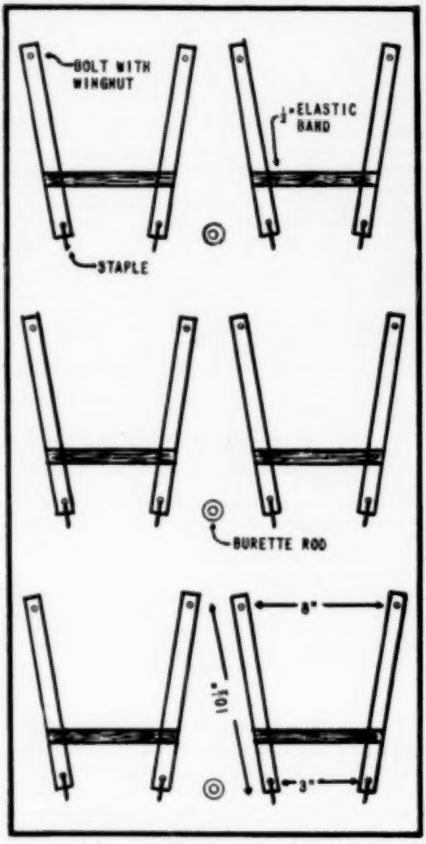

Fig. 1.—Schematic drawing of table top 25 in. x 48 in. accommodating six pigeons.

The iron strips are fixed at one end with a $^3/_{16}$-in. flat staple to act as a hinge and the other end fastened through a bolt equipped with a wing nut. A half-inch elastic band is placed over the two iron strips as shown to secure the pigeon legs.

The pigeon wings are spread taut under the iron strips, tightened in place with the wing nut, and the legs secured under the $^1/_2$-in. elastic band as shown (Fig. 2).

Fig. 2.—Photograph of a pigeon immobilized and cannulated for assay.

The immobilization of the birds by the above method reduces to a minimum the danger of having cannula torn from the vein by sudden movements of the pigeons.

We have found that the cannulation forceps described by Haas and Goldblatt (2) are a useful tool for rapid and deft cannulation of the alar vein of pigeons. The forcep tips were bent at right angle and fashioned to thin needle-like prongs, such that both tips when held together would fit readily into the lumen of a 20-gauge needle. The alar vein wall is pierced with the needle-like tips, the prongs separated and the cannula, a blunt 1-in. 20-gauge hypodermic needle, inserted and tied *in situ*.

By the above-described technique, six pigeons can be secured and cannulated within a short period. An assay using at least six pigeons each on a reference standard and test material can be completed within a working day.

* Received May 29, 1950, from the Research Laboratories, Carroll Dunham Smith Pharmacal Co., New Brunswick, N. J.

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Book Reviews

Biologie der Goethezeit. Edited by ADOLF MEYER-ABICH. The Chronica Botanica Co., Waltham, Mass., 1949. 302 pp. Illustrated. 15.5 x 23.5 cm. Price $5.50.

This book by the well-known German pioneer of a new philosophy of nature, Adolf Meyer-Abich, is much more than another book on Goethe caused by the occasion of the two-hundredth anniversary of the poet's birthday and devoted to his memory. It uses Goethe as a striking beginning of, and example for, the new philosophy, and the occasion as a vantage point for bringing this philosophy to the attention of a greater audience.

What then is this new philosophy of nature? It is based on the "*Gestalttheorie*," i.e., the idea that everything in existence represents an entity the parts of which are determined by the constructive idea underlying the specific and unique form of the given entity. There is no doubt of the Aristotelian element in this theory which its main representative, Wertheimer, formulated as follows:

"The given matter is in itself 'formed' to a different extent. Given are more or less constructed, more or less definite, entities and entity processes, with often very concrete entity qualities, with inner regularities, with significant entity tendencies, with entity determined influences for the individual parts."

The author states in his excellent introduction that "Goethe's research in natural science is, fundamentally, that of [Greek] antiquity, oriented, however, in the direction of modern dynamic concepts." And he adds that "Platon or Aristoteles would have done their research in exactly the same way, if they would have occupied his [Goethe's] place in intellectual history."

It is significant that the excerpts from Goethe's publications on natural science concepts and facts are taken from his writings on morphology (philosophy of morphology and general morphology). The idea of the "idealistic morphology" comes, indeed, already in its term (and naturally still more through the central place given to it by Goethe) very close to the constructive sense relationship between an entity and its parts on which modern natural philosophy, as represented by Meyer-Abich, is based.

The excerpts from Goethe's writings are followed by reprints of respective articles by Goethe's contemporaries. (Georg Forster, Alexander v. Humboldt, Lorenz Oken, Carl Gustav Carus, Karl Ernst v. Baer, Johannes Müller.) It has to be kept in mind, however, that of these men only the two first names really lived in approximately the same time as Goethe while the others were much younger and, surviving him by many years, developed into a realm of much extended knowledge.

Meyer-Abich closes his book with a chapter entitled "Goethe's principle of compensation, the first holistic fundamental law of modern biology," the word "holistic" being coined by the South African marshal and philosopher, J. C. Smuts, from the Greek "to holon" meaning the whole. (See Smuts, J. C., "Holism and Evolution," London, 1926.) It is Goethe's statement that "there cannot be anything added to one part of an entity without deducting something from another part and vice versa" which Meyer-Abich calls the law (or principle) of compensation and which he considers "the lasting bequest of Goethe's morphology to modern biology." This chapter contains furthermore a very interesting discussion of the modern "vitalism" of Driesch and of Vernadsky's modern "geochemistry."

The book of Meyer-Abich appeals not only to readers interested in the phenomenon Goethe, but to everyone interested in the phenomena of the natural philosophy of our time.—GEORGE URDANG.

The Chemistry of Industrial Toxicology. By HERYEY B. ELKINS. John Wiley and Sons, Inc., New York 1950. ix + 406 pp. Illustrated. 14 x 22 cm. Price $5.50.

The author of this book has recognized the need for a book on industrial toxicology with a nonmedical approach and has, therefore, made no attempt to characterize completely the physiological conditions resulting from exposures. He has presented, however, a well-organized introduction to toxicology for the chemist and engineer, thus giving these workers the tools they need for recognizing hazards and maintaining the necessary chemical control.

The section on evaluation of hazards should enable an unexperienced chemist to proceed logically and with little lost motion to make plant surveys for hazards. There is a large section on specific toxic agents, describing methods of evaluation, type of damage and maximum allowable concentrations. The type of damage to the body is usually presented, though briefly.

Preventive measures are discussed in the order in which they usually should be considered. The instruments used for sampling and measuring toxic agents are described and their applications are given. Specific analytical methods for determination of the toxic agents are also included.

The reviewer believes that the author has successfully produced a book that will give the chemist or engineer the information needed for common industrial toxicological problems.

Fundamentals of Physical Chemistry for Premedical Students. By H. D. CROCKFORD AND SAMUEL B. KNIGHT. John Wiley and Sons, Inc., New York, 1950. xi + 366 pp. Illustrated. 14 x 22 cm. Price $4.25.

Many schools now offer a short course in physical chemistry for premedical, biology, and other students not chemistry majors. Also, some schools of pharmacy have introduced such courses into their curricula, and many others are considering such action. These facts make the appearance of this book quite important. It offers a survey of the topics of

physical chemistry giving less detail in each topic than those books designed for longer courses, and it involves no mathematics beyond college algebra.

The chapters of the book are written in such a way that the student will find little difficulty in following development of the subjects. The introductory chapter is very simple, offering a review of the elementary physical principles that are learned in general chemistry and presenting the purpose and importance of physical chemistry. The topics are then arranged by chapters in the usual order. The treatment of gases and solutions seems adequate, and the significance of *p*H and its measure is also given the emphasis that is probably desired for students of biology and medicine. A simple introduction to colloids is included, but the instructor may find it necessary to add more specific information on proteins. The section on radioactivity seems entirely too brief, giving too little background information when one considers the probable contact with radioisotopes that the student faces in the future. X-rays are not discussed. There are occasional omissions that may prove disturbing to the instructor; for example, after a discussion of orders of reactions and of catalysts the authors include a section on enzymes which answers none of the questions of the student as to order of reactions catalyzed by enzymes, effect of inhibitors, etc. In some instances the authors are forced to present final equations without any derivation to avoid use of the calculus but fail to use other devices which may illustrate the validity of the equations. For example, no graphs are shown to illustrate reaction orders, and the final equations are simply presented for the students' acceptance. Thermochemistry is not included in the text.

It must be recognized that a text intended for a short course in physical chemistry must necessarily leave out many topics and must limit its discussion very severely. It would be expected that instructors may differ as to which topics could properly be omitted, and, to be sure, most teachers will want to add some topics to supplement the text.

The authors of this book have used a very valuable device in development and application of equations—by including in the expressions the units of each component and treating these units mathematically along with the figures, they make clear the units of the constants derived. This device should be welcomed by student and teacher, and should prove useful in the solving of problems.

References are not to original journal articles but to books which treat the subjects in more detail. This appears to be a good system, since the references will probably be adequate for the student and since it should be possible for the library to furnish all references required and keep them available for the convenience of the student.

The problems seem to be well designed to cover the subject at the right level. Answers are included in the appendix. Review questions are also included, and these should assist the student in study.

It is the reviewer's opinion that this book will prove a practical and acceptable textbook, particularly from the students' viewpoint. It deserves scrutiny by any teacher of a short course in physical chemistry. For those faculties of pharmacy schools debating the value of nonmathematical physical chemistry it will answer many questions.

Organic Chemistry. By PAUL KARRER. 4th English ed. By H. V. SIMON and N. G. BISSET. Elsevier Publishing Co., Inc., New York, 1950. xxi + 973 pp. Illustrated. 17 x 26 cm. Price $8.50.

The fourth English edition of this book has the same excellent character as previous editions. It has been expanded to include many new compounds. The same general organization is maintained, and the new edition differs from the older ones chiefly in having added the newer methods and the more important new compounds. As with the earlier editions, this book is an excellent reference text, and it will continue to occupy an important position on the bookshelf of the organic chemist.

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The Actinomycetes. (Annales Cryptogamici et Phytopathologici, Vol. 9). BY SELMAN A. WAKSMAN. Waltham, Mass.: The Chronica Botanica Co.; New York City: Stechert-Hafner, Inc. xviii + 230 pp. Illustrated. 15 x 24 cm. Price $5.

Principles of Ionic Organic Reactions. BY ELLIOT R. ALEXANDER. John Wiley and Sons, Inc., New York, 1950. viii + 318 pp. Illustrated. 15 x 24 cm. Price $5.50.

General Chemistry. BY JOHN ARREND TIMM. McGraw-Hill Book Company, Inc., New York, 1950. xii + 764 pp. Illustrated. 15 x 23.5 cm. Price $4.50.

A Laboratory Manual for Inorganic Pharmaceutical Products. BY ROBERT F. DOERGE AND CHARLES O. WILSON. Hemphill's, Austin, Texas, 1950. viii + 191 pp. 22.5 x 27.8 cm. Price $2.95.

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Surface-Active Quaternary Ammonium Germicides. By CARL A. LAWRENCE. Academic Press, Inc., New York, 1950. vii + 245 pp. Illustrated. 15.5 x 23.8 cm. Price $6.

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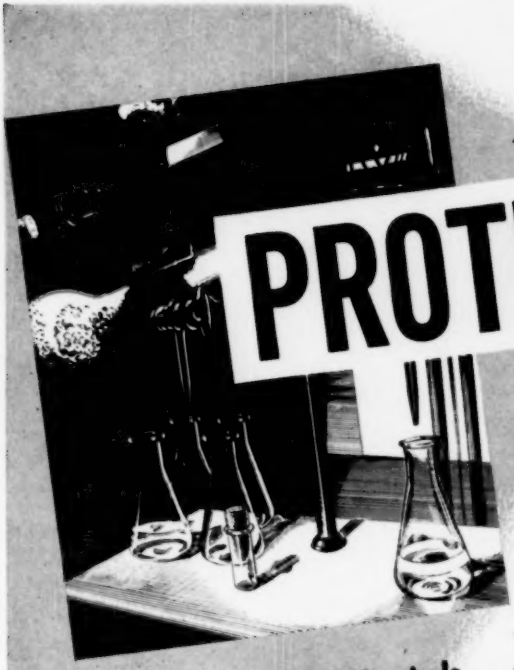

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